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A. SHARPLES

young rubber. The 'die-back' of 4-12-month old trees wherein the top wilt, the green bark turns black with the appearance later of three to five different caulicolous fungi, finally resulting in death, is apparently attributable to lightning. The affected trees occur in patches and when cut back to stump height regenerate rapidly with a discontinuance of the damage. The influence of lightning on the growth of trees, if the trees are not killed outright, usually takes the form of a modification of the developing wood cells of the season so that the continuity of the normal structure of the wood is broken and a 'lightning ring' is formed."

Recent observations prove that lightning effects in rubber plantations are quite as definite as those in coconut plantations and strongly support the observations made and conclusions arrived at by the investigation of the causation of diseases of coconut palms.

The observations on the effects of lightning on rubber plantations can be most conveniently treated under two headings:

- (a) Lightning effects and "die-back" usually found in young trees
- (b) Lightning effects and "claret-coloured bark canker at the collar" found on trees from 4 to 20 years of age.

Lightning effects as in (a) above. The symptoms shown by young rubber trees affected by lightning are well described in the quotation already given. The important point is that few losses occur, for young trees are seldom killed outright and when cut back to stump height, the cut being made through healthy tissues, regeneration is rapid, the growth being continued by the shooting of lateral buds from the unaffected tissues. Careful examination of the root systems of young trees affected by lightning show that they remain healthy; a root examination is necessary to make certain that the symptoms are not the result of an attack by one of the usual root disease-causing fungi.

The following is a record of an area recently affected by lightning. The area was planted in 1928 and budded in December, January, 1929-30. There were two areas affected, a large area situated on an exposed hilltop and a small area on the side of a hill, one mile distant.

The lightning storm, which occurred on November 3rd, 1931, was notably severe one. Nothing unusual was seen until November 11th, 1931 when over 100 trees were found showing the symptoms described above (Plate I, fig. 2). The writer was notified on November 11th, 1931 and inspected the area on November 13th, 1931; on this visit a careful root examination was undertaken, and this showed the root systems to be perfectly healthy. In the large area 121 trees were treated; of the

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number eight had to be cut out completely, the rest were treated successfully by pollarding. The number of treated trees in the smaller area was twenty, of which five were total losses.

The record is similar to that described for large affected areas of coconuts in the previous section and the diagnosis of lightning strike could be made with confidence. The next record will also show a very considerable and probably significant degree of similarity to the records from coconut areas affected by lightning and is especially interesting from the point of view of delayed effects.

Lightning effects as in (b) above. This case of lightning injury on rubber trees, 4-5 years old, was investigated in March, 1931. The trees were planted on a hilly slope on contours; the ground was covered by a thick cover crop of *Centrosema pubescens*.

A lightning storm in close proximity was noted 5 days before the affected trees were found. Trees on two contours were affected; on the lower contour only two dead trees were found, but on the upper, one dead tree and in addition seventeen neighbouring trees, all slightly affected at soil level, were observed.

The dead trees were taken out immediately. The seventeen trees, presumably slightly affected by lightning, showed discoloured cortical tissues at ground level. This discoloured tissue formed a patch about 6 in. square and extended through the thickness of the cortex. The discoloured patches of cortical tissue showed symptoms exactly similar to those described for the cortical disease of *Hevea*, long known as patch canker or claret-coloured bark canker. A discoloured patch was stripped from the wood and isolations were made in the laboratory in various ways. Thirty hours after setting in the culture media a profuse growth of white mycelium was evident, which ultimately proved to be a species of *Pythium*.

The obvious point is, that if the trees with the bark attack at the collar had remained unnoticed at the time the dead trees were taken out, and had been left untreated, there would have been a peculiar outbreak of root disease reported a few months later, for which it would have been difficult to provide the correct explanation. This is a parallel case with the delayed effects observed in the coconut palm investigations. Later investigations, described below, provide further evidence showing that the discoloured tissue at the collar is typical of claret-coloured bark canker.

Before describing other recent interesting cases of the association of lightning effects on rubber trees and claret-coloured bark canker at the

collar a few remarks on the disease may be of interest. This disease, known variously as claret-coloured bark canker, purple canker or patch canker, is a well-known disease of *Hevea brasiliensis* and was first discovered on this host in 1903. Petch records that it has been found in Java, Sumatra, and Fiji, and that it is said to be of rare occurrence in the Federated Malay States. The following remarks, with reference to this disease, are taken from the same authority (6):

"The most serious cases of claret-coloured bark canker are those in which the tree is attacked at the collar. The disease may then run rapidly round the base of the tree and kill it in a few weeks.

"Bark attacked by claret-coloured canker has a peculiar smell, which soon attracts boring beetles, particularly a small brown beetle about the size of the shot-hole borer. When the disease has been in progress for a few weeks, the decayed patches are usually riddled with this borer.

"The *Phytophthora* which causes claret-coloured bark canker is identical with that which causes the similarly coloured canker in cacao."

The cases of claret-coloured canker in Malaya recently found associated with trees affected by lightning are invariably those in which the tree is attacked at the collar. The chief danger in such cases is that boring beetles, which are attracted by the peculiar smell of the affected tissue, may enter the tree, and if this happens, such trees succumb in the majority of cases.

With reference to the fungus causing the symptoms, the position was not clearly understood until 1929, when Thompson (13) showed that two species of *Phytophthora* and one species of *Pythium* are direct causes of patch canker in Malaya; further, that seven other species of *Phytophthora* isolated from host plants other than *Hevea* are capable of causing patch canker symptoms if artificially inoculated into the bark of rubber trees. Thus it seems obvious that more than one species of *Pythium* or *Phytophthora* may be found to be involved in the production of the diseased claret coloured, cortical tissue which has been found at the collar of trees affected by lightning.

The most noteworthy occurrence of the association of lightning and claret-coloured bark canker at the collar can now be described. During the investigations on lightning effects the writer has noted the dates of lightning storms occurring in the vicinity of Kuala Lumpur. Two heavy thunderstorms were noted on November 18th and 19th, 1931; both took place between the hours of 1.30 and 4.30 p.m. On December 2nd, 1931 a report of lightning damage was received from an estate only 3 miles from the Rubber Research Institute. A visit was made and several lightning

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patches were found on trees 20 years of age. There was no cover crop present.

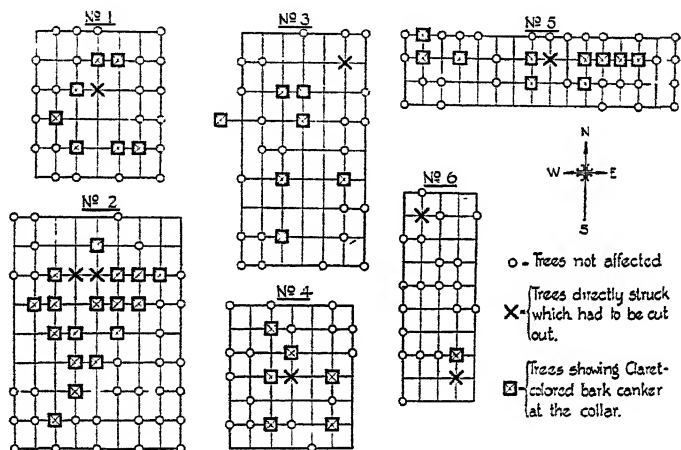


Text-fig. 2. Plan of estate. Showing position of lightning-strike patches (o).

Text-fig. 2 shows the patches situated on a direct north and south line. The distribution of the affected trees in each block is shown in

Text-fig. 3. The total number of affected trees was 56. Of these eight were killed outright and 48 were treated for claret-coloured bark canker at the collar.

The symptoms shown by the affected trees could not be mistaken. The badly affected trees which had to be cut out were killed as a result of the scorched cortical tissues being rapidly invaded by the *Diplodia* sp. which is the cause of "die-back" in rubber trees. This black, discoloured cortical tissue proves attractive to boring beetles, and the rapid penetration of these insects into the stem results in the early death of the tree. The borer attack on badly affected trees is of importance when considering treatment of the slightly affected trees, which again showed typical symptoms



Text-fig. 3. Showing distribution of affected trees.

of claret-coloured canker at the collar, because, as mentioned above, such affected cortical tissue attracts boring beetles, and on this account it is imperative to remove it as quickly as possible to prevent penetration by the insects.

The slightly affected trees all showed the typical symptoms of claret-coloured bark canker at ground level, in greater or less degree. Plate II, figs. 3 and 4, shows the appearance of an area of discoloured cortical tissue, 10 in. by 5 in., which was stripped from the wood at the collar of one tree. Fig. 3 shows the extent of the discoloration of the affected area when the outer bark layers are scraped away. Fig. 4 shows the appearance of the inner surface of the affected cortical tissues; this surface is directly in contact with the wood, and a reflection of this appearance is

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found on the wood surface. The white patches are pads of coagulated latex which has infiltrated from the attacked cortical tissues; these rubber pads lie in shallow depressions on the inner surface, which are formed as a result of the pressure set up.

The photographs illustrate an extreme case in which a comparatively large bark area is affected, with the fungus penetrating to a slight depth into the wood beneath. The more numerous cases are those in which a smaller patch of cortical tissue is involved, and though the wood surface beneath is discoloured, there is no penetration of the woody tissues by the fungus.

The treatment of the trees showing the small patches of diseased tissue at the collar is simple. The area affected is delimited by slight scraping with a chisel or similar instrument; when this has been accomplished, the diseased area with about 1 in. of surrounding healthy tissues is stripped from the wood. The only warning note is that as little scraping as possible should be done to prevent diseased tissues becoming mixed with the soil. There is no danger if the stripping of the whole diseased area is done so that it comes away in a single piece. The diseased tissue should be destroyed and the exposed wood surface painted first with a suitable disinfectant and later covered with a permanent wound dressing.

The *Pythium* sp. isolated from the diseased cortical tissues is being studied and will be reported on subsequently.

DISCUSSION.

A few comments are required on some of the general features observed during this investigation. When the work was commenced the evidence provided by the available literature led to the conclusion that an association of a *Phytophthora* sp. with the common bud-rot symptoms of palms would be found. No evidence has been obtained to support this conclusion.

This finding is thrown into greater prominence because of the close association found between lightning effects in rubber plantations and the secondary symptoms of claret-coloured canker produced by a species of fungus closely related to the genus *Phytophthora*. The effects of lightning in rubber and coconut plantations are quite opposite in one respect. In rubber plantations the secondary symptoms are caused by fungi which have been long known as probable causes of specific diseases of rubber trees. On the other hand, the secondary bud-rot symptoms in Malayan coconut plantations cannot be connected up with any disease-causing fungus. *Marasmius palmivorus* Sharples simply accelerates defoliation and has nothing to do with the actual rotting of the bud tissues.

There is nothing unexpected in the association between lightning and the *Diplodia* sp. commonly found attacking rubber trees suffering from "die-back." The partiality of this fungus for scorched cortical tissues has been proved (15), and this feature has been amply demonstrated in trees scorched by lightning which have been examined during the present investigation.

The occurrence of the *Pythium* sp. which causes the claret-coloured canker at the collar of rubber trees slightly affected by lightning is difficult to explain. The difficulty might be overcome if claret-coloured bark canker of the tapping panel was a common disease in Malaya, as it is reported to be in Ceylon. This disease of the tapping panel is rarely met with in Malaya, so this cannot be considered as a source from which the *Pythium* sp. would be derived. This fungus might escape notice on areas carrying a heavy cover crop, for the cover plant would provide the desirable humid conditions for growth and spread, and it may be submitted that the fungus might be present without special symptoms becoming prominent on the cover crop. If evidence was forthcoming to support this suggestion an acceptable explanation of the observed phenomena could be provided. But in several mature areas recently found affected by lightning, where the slightly affected trees were all found to be suffering from claret-coloured bark canker at the collar, no cover crop was present, so that this suggestion cannot be accepted.

Two alternatives are available:

- (a) That the organism is a soil-inhabiting fungus.
- (b) That the fungus is commonly present in the interstices of the bark, where it may be able to live saprophytically and later may be washed down to the collar during heavy rains.

No evidence has been obtained, as yet, to support either alternative.

Comment has often been made in Malaya upon the relationship between bud-rot of coconut palms and the presence or absence of various species of *Phytophthora*. Thompson (13), in his comprehensive work on Malayan species of *Phytophthora* found associated with diseases of the cortical areas of the rubber tree, remarks: "Bud-rot of the coconut palm caused by *Phytophthora* has not been found in Malaya, but whether this is due to the fact that typical members of the cacao group of *P. palmivora* Butl. are not present is a matter for conjecture. Inoculations on coconut palms with the local forms of *P. palmivora* Butl. from *Hevea* have given negative results, while an inoculation with No. 10 was successful. No. 18 (Ashby's strain from coconuts in Jamaica) is a member of the

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rubber group of *P. palmivora* Butl. This strain also failed to produce bud-rot symptoms when inoculated into coconut palms in this country, but the result is inconclusive owing to the dry-weather conditions which prevailed at the time. It is hoped to experiment further with inoculations on coconuts and other palms when occasion permits, in order to obtain more information as to the degree of virulence of the members of the cacao and rubber groups of *P. palmivora* Butl. on these hosts. Coconut plantations and rubber plantations are often located together, and in most native rubber holdings coconut palms can be found. Yet, in areas where black stripe disease (caused by *P. palmivora* Butl.) is prevalent, adjoining coconut palms remain free from bud-rot."

These remarks fairly summarise the present position. All efforts to demonstrate a connection between bud-rot symptoms and a fungus of the *P. palmivora* Butl. group have proved a failure. It is unlikely that such failure could be attributed to faulty technique, in view of the fact that work on the same lines resulted in the successful isolation of the *Pythium* sp. causing claret-coloured bark canker on rubber trees slightly affected by lightning. Further, during the year, two other forms of *Phytophthora* have been successfully isolated, so that the conclusion can be drawn safely that the only reason why a *Phytophthora* sp. cannot be obtained from bud-rot of palms in Malaya is that no form of *Phytophthora* is present and therefore need not be considered a factor in the problem at the present time.

The conclusion regarding the absence of a *Phytophthora* sp. in the Malayan bud-rot problem is a perfectly sound one, but the position may change in the future. The recent culture work of Leonian⁽⁴⁾ proves conclusively that variants of a *Phytophthora* sp. may be produced from a parent type by dissociation. He further points out that similar variations may be expected to take place in nature, for under the more complex natural conditions there is a possibility of an infinite number of changes of growth conditions as compared with those which may be encountered in an artificial culture medium. Thus it may well be that future observations will result in a *Phytophthora* sp. being found as a causal agent in connection with bud-rot of palms in Malaya.

SUMMARY.

1. This investigation proves that lightning is a factor of the first importance in the causation of disease on coconut palm plantations in Malaya.
2. The "bud-rot" symptoms occurring in Malayan coconut palm

plantations are produced entirely by lightning or the after-effects thereof. No evidence of the presence of a *Phytophthora* has been obtained.

3. Lightning has been proved to be of some importance in the causation of disease on rubber plantations.

4. The typical effects on rubber plantations are described, and attention is specially directed to the association of claret-coloured bark canker at the collar of trees slightly affected by lightning.

The writer wishes to acknowledge the considerable help rendered by his colleague, Mr H. Gunnery, in the preparation of photographs and recording of field work. Thanks are also tendered to Mr H. T. A. Biddlecombe for great assistance in the coconut investigations and to Mr G. Shelton-Agar for similar assistance in connection with the work on rubber plantations.

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EXPLANATION OF PLATES I AND II.

PLATE I.

Fig. 1. Typical appearance of coconut area affected by lightning.

Fig. 2. Young rubber trees affected by lightning.

PLATE II.

Fig. 3. Area of discoloured cortical tissue at collar of a 20-year-old rubber tree slightly affected by lightning.

Fig. 4. The inner face of the discoloured cortical tissue shown in Fig. 3. Note coagulated rubber pads.

(Received April 19th, 1932.)



Fig. 1.



Fig. 2.

SHARPLES.—LIGHTNING STORMS AND THEIR SIGNIFICANCE IN RELATION TO DISEASES OF
(1) *COCOS NUCIFERA* AND (2) *HEVEA BRASILIENSIS* (pp. 1-22).



Fig. 3.



Fig. 4.

SHARPLES.—LIGHTNING STORMS AND THEIR SIGNIFICANCE IN RELATION TO DISEASES OF
(1) *Cocos nucifera* AND (2) *Hevea brasiliensis* (pp. 1-22).

A SULPHUR-DEFICIENCY DISEASE OF THE TEA BUSH

BY H. H. STOREY, M.A., PH.D.

(*East African Agricultural Research Station, Amani*).

AND R. LEACH, B.A., A.I.C., T.A.

(*Department of Agriculture, Nyasaland Protectorate.*)

(With Plates III-VI.)

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IN recent years an obscure disease of the tea bush, to which the name *Yellows* has been applied, has seriously handicapped the production of tea in Nyasaland. In 1927 it was characterised by Dr E. J. Butler(2) as "unquestionably one of the most serious diseases to which the tea bush is liable." The cause of the disease and any means for its control have remained hitherto unknown.

We now present evidence that this disease is the result of a deficit of sulphur in the bush, relative to other necessary elements. It can be prevented and usually can be cured by supplying sulphur in an available form to the bush.

This conclusion is the outcome of co-operative studies, begun in 1929, during a two months' visit to Nyasaland by one of us (Storey). The

field experiments in Nyasaland were carried out by Leach and the pot and water-culture experiments by Storey at Amani. A second visit to Nyasaland was paid by Storey in February, 1932. For the design of all experiments and for the conclusions drawn from them the authors are jointly responsible.

I. HISTORY OF THE DISEASE IN NYASALAND.

This disease was first reported in 1926 by Smee (26), although it was undoubtedly prevalent in earlier years. On the basis of symptom manifestation he identified it with the root disease attributed by Petch (21) to the attack of *Botryodiplodia theobromae* Pat. Butler (2), during a short visit to Nyasaland in 1927, formed the opinion that the disease was not initiated by *Botryodiplodia theobromae* or by any other fungus. He was unable to find a cause, but suggested the possibility of a virus infection.

Our first step in the investigation of the disease was to test the virus hypothesis. Experiments directed to this end gave it no support, although they were not carried to the point where the evidence was conclusively negative. In the meanwhile, however, we had obtained evidence which caused us to suspect a soil deficiency. At first our suspicions rested upon magnesium, since Hornby has reported that tobacco may suffer from a deficiency of this element in Nyasaland (14). Our experiments, however, soon caused us to discard this explanation of yellows disease in favour of one based on sulphur deficiency.

The hypothesis that yellows was a root disease was revived in 1930, when Dr W. Small (25) demonstrated that *Rhizoctonia bataticola* (Taub.) Butler was almost invariably present in the roots of dead or dying bushes. Our observations upon the relation between this fungus and the disease are reported later; they give no support to the idea that the disease may be initiated by this fungus.

II. SYMPTOMS OF THE DISEASE.

The affected tea bush passes through a characteristic degeneration ending in the death of individual shoots and branches, and finally of the whole bush. In the early stage there is little or no reduction in size of the leaves or in length of the internodes, but the leaves become mottled with yellow, the network of veins remaining green (Plate III, fig. 1 and Plate IV, fig. 4). As the disease advances the reduction in the size of the leaves and in the length of the internodes becomes marked. The mottling of the leaves gives way to a general severe chlorosis (Plate III, fig. 2 and Plate IV, fig. 5). The leaves tend to be somewhat uprolled and have a peculiar

stiff texture, which causes them often to break when bent. Frequently the leaf tissue dies from the tip or edge, shrivelling to a dark brown colour. With the further development of the disease these changes become more pronounced. The reduction of the leaf size may be extreme, mature leaves sometimes being no longer than 1.5 cm., while the shoot is thin and weak with closely crowded nodes. Meanwhile all leaves formed after the disease appeared, except the youngest, are shed (Plate III, fig. 3 and Plate IV, fig. 6). Lateral buds make premature development, producing stunted shoots with minute yellow leaves. Eventually the terminal bud of the shoot dies and a gradual die-back of the shoot sets in. The final state is a leafless bush bearing many dead or dying thin shoots, although occasionally living axillary shoots may be found near the base (Plate IV, fig. 7).

The disease rarely progresses uniformly in the several branches of a bush. The bushes illustrated in Plate IV, figs. 4-7, are exceptional, having been specially selected as uniformly diseased, for the sake of clearness. Usually some branches of a bush are severely diseased, while others are mildly diseased or even healthy. Occasionally an extreme unilateral effect may be seen, where one half of a bush is severely diseased and the other half healthy.

The symptom picture which we have described is a characteristic one, and we have felt justified in assigning it to a single cause. Our experiments show that in a number of localities, if the hypothetical cause—sulphur starvation—be removed, the symptoms disappear. It is, nevertheless, conceivable that some other slowly acting cause may produce similar symptoms. If this be so, we have encountered no instance of it in the course of our studies. The symptoms that we have described differ from the sudden wilting characteristic of a rapidly acting root disease—such as that attributed to *Armillaria mellea* (Vahl.) Sacc.—and from the chlorosis due to general starvation.

The root of a diseased plant may be poorly developed. This is most readily seen in young diseased plants which have developed from seed planted in the field at stake and have in consequence grown without disturbance of their roots. The difference lies in the number and spread of the lateral roots and in the growth in thickness of the taproots. In an old bush, which has already formed an extensive root system, we have observed no effect upon the form of the roots consequent upon the onset of yellows disease. Butler⁽²⁾ has reported that the bark and wood of the root of a diseased plant tend to separate more easily than in a healthy root, and that the wood cuts more easily. We have been unable to con-

vince ourselves that this character is invariably associated with the disease. The starch reserves of the root, as revealed by an iodine test on the cut end, are depleted in the diseased plant. A severely diseased plant usually contains little or no starch in its roots.

An histological examination of the small yellow leaves of an affected shoot suggests that their development was arrested while they were still immature. A healthy leaf contains a palisade layer of about three cells thickness, and a mesophyll below, the individual cells of which are separated by large air spaces. In the diseased leaf the palisade layer is poorly differentiated, frequently only a single layer of cells being elongated; the mesophyll cells are crowded and more uniformly arranged, the intercellular spaces being much reduced. The cell walls are thinner in the diseased than in the healthy leaf. The stomata of the lower surface of the diseased leaf are more numerous than on the mature healthy leaf, their frequency in a diseased leaf being similar to that in an immature healthy leaf of about the same size. In the early stages of the disease the plastids are reduced in size and number, while ordinary histological methods reveal none in a severely diseased leaf.

A few analyses of material from diseased and healthy tea bushes have been carried out¹. The results are open to criticism on various grounds, particularly in respect of equivalent sampling. The figures for four pairs of samples of the terminal portions of shoots, consisting of the bud, one or two young leaves and a short length of young stem, showed a significantly greater sulphur content in the healthy material than in the diseased.

III. THE CLIMATE AND SOILS.

The main tea-growing area of Nyasaland is confined to a narrow belt along the southern side of the Mlanje Mountain massif, at an elevation averaging 2500 ft. The highest rainfall, amounting to an average of 88 in. per annum, is encountered generally close to the scarp; it decreases rapidly as the distance from the scarp increases. The area suitable for tea is thus restricted on the north by the steep slopes leading up to the mountain face and on the south by the falling off of the rainfall below about 60 in. per annum. Of the total rainfall about 74 per cent. falls in the five summer months, December to April. The following description of Mlanje soils has been supplied by Capt. A. J. W. Hornby, Soil Chemist in the Nyasaland Department of Agriculture.

The soil groups to which the majority of Mlanje soils on which tea is grown belong are those of the lateritic loams and ferruginous laterites. The distinguishing characters

¹ Analyses by Dr R. R. Worsley and Mr W. E. Calton, Amani.

of the soils are largely influenced by the amount and distribution of the precipitation [see above], and they are generally well developed. Under the heavy precipitation accompanied by the temperatures usually met with, it is expected that the soils would be considerably leached and the rocks from which the soils were derived would have been very completely decomposed. This is borne out by numerous examinations of Mianje soils. The percentage of alkalis and alkaline earths is low, and the composition of the clay fraction usually points not only to the presence of free aluminium hydroxide but to the ratio of silica to alumina which is accepted as distinguishing a lateritic soil or a laterite (16).

The colour of the soils varies from a dark brown or grey to chocolate and terracotta. The factors influencing the colour are largely the amount of slope and the surface movement to which they have been subjected in the past, the composition of the parent rocks, such as syenite, and the type of vegetation originally present. The three main soil series belonging to the two main groups can be distinguished as dark grey to dark brown friable soils formed under the highest rainfall, and two red compact soils one of which is characterised by a horizon where accumulation of concretions of hydrated iron and alumina goes on.

The dark grey or dark brown soils of the first series in the natural state are distinguished by a very high content of nitrogen and organic matter in the A horizon, good absorption, and very low amounts of potash, lime and magnesia. All soils dealt with are carbonate-free but do not indicate a high lime requirement. The specific acidity is normal for a tea soil and increases down to a depth of about 18 in., where a decrease commences and continues to considerable depths. Little change can be observed in the granular structure of this B or illuvial horizon, the colour of which is reddish until rotten rock is met with. Where such soils are found on steep slopes, the B horizon is shallow, the composition is variable and some are hardly distinguishable from the colluvial soils formed near the mountain massif by soil creep and movement by rain wash.

The red soils characterised by large accumulations of iron and alumina compounds at certain horizons have been formed on the flatter lands under an average rainfall of about 70 in. per annum. They are generally compact soils, the organic matter however being a larger fraction of the humic layer in the A horizon than the colour indicates and having a larger influence on the somewhat inferior physical properties. The surface soil might be termed cemented when organic matter is absent. Often the accumulations in the B horizons form a network or a crust of iron compounds, more or less continuous, which may be almost impervious to surface water and leads to seepage at lower levels. This often occurs in gullies formed by comparatively recent denudation and erosion. Naturally at certain points leaching is very extensive and with water-logging may be the initial cause of certain crops suffering from various starvation diseases (15). The main distinguishing features of the profiles of the soil types examined are the increase in specific acidity, stickiness and compactness in the A horizon to a depth of from 30 to 50 in. At a depth of about 3 ft. on the average gentle slope a drop in specific acidity commences. This is generally the depth at which the B horizon starts. The acidity in the B horizon continues to decrease as in the former soils and often reaches a point which is considered too low for the ideal tea soil. The C horizon may only be found at great depths and is generally characterised by softened orthoclase, hornblende and angular quartz. Layers of kaolin may be met with. The soils are ferruginous laterites.

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The third series of soils comprises most of the soil types on which tea is grown, and, being formed under a comparatively low rainfall of about 60–68 in., are similar but superior soils to those of the Cholo and Zomba series which have been the subject of extensive study (17). The content of organic matter is better than that present in the Cholo soils both in quantity and condition, but it is apt quickly to deteriorate under cultivation. The soils are, as with the ferruginous laterites with concretions, most subject to erosion and require very careful management both with manuring, cultivation and terracing. They vary considerably according to the slope, leaching varying according to the run-off.

On the outskirts of the Mlanje tea-belt proper, types of immature red loams and true red loams occur. These need not concern us here any more than the local soils which may be formed under the influence of abnormal movement of soil water during the wet season.

In the following pages reference is made to the soil series described above under the numbers I, II and III.

A secondary tea area has been recently developed in the neighbourhood of Cholo Mountain, at an elevation of about 3000 ft. Here the rainfall varies from about 48 to 56 in., of which 75 per cent. falls during the five months of summer (17). The soils of this area resemble generally the Series III Mlanje soils, and have been fully studied by Hornby (17).

Analyses of seven Mlanje soils for total sulphur content gave a mean value of 0.031 ± 0.010 per cent. sulphur¹. This value is within the range encountered in normal soils, *i.e.* 0.015 – 0.06, according to Powers (23). All the soils were taken from tea gardens, or the vicinity of tea gardens, where yellows disease was prevalent. There was no consistent relation between the total sulphur content and the condition of the bushes at the actual site of sampling.

IV. THE DISEASE IN THE FIELD.

Tea bushes affected with yellows disease occur in almost all the gardens of the Mlanje and Cholo areas of Nyasaland. Their frequency varies greatly between different gardens, but in the majority this disease is an important factor in limiting production.

In relation to the incidence of the disease, the soils of Series I need to be considered apart from the red soils of Mlanje and Cholo. In the former, yellows disease is liable to appear early in new plantings on virgin soil; in the latter, yellows generally only follows a loss of original fertility through cropping, erosion, or some other cause.

The spontaneous appearance of yellows in young plantings of tea in newly cleared soils of Series I, indicates that the conditions that produce yellows are already present before agricultural operations are under-

¹ Soil analyses by Mr G. Milne, Amani.

taken. Planters' experience shows that those bushes that survive the initial disease phase generally make good growth subsequently. The importance of the disease during early growth may be judged from a count made in a typical garden; at the end of the first year's growth the incidence of the disease averaged 36 per cent. We may mention, in contrast, that in a similar garden where the young tea at the time of planting on virgin soil received a dressing of a fertiliser containing a sulphate, the bushes were free from yellows disease.

On the red soils (Series II and III) yellows disease is usually unimportant until a deterioration of the soil has occurred subsequent to the initial clearing from bush. The deteriorating influences appear to have been primarily erosion, prolonged cropping and competition. Thus the disease is prevalent on badly surface-washed slopes (Plate IV, fig. 9), on the sides of drains and roads, on lands that have previously borne other crops than tea and particularly on the sites of native gardens, along forest margins, often around the bases of large shade trees (usually *Albizia* spp.) in old established gardens, and in neglected gardens where weed growth has remained insufficiently checked. On the other hand the disease is least in evidence in valleys that have received the soil washed from the hill-sides (Plate IV, fig. 9) and in newly cleared land protected from severe surface erosion. Bushes growing on ant-hills rarely develop yellows, although often growing poorly and suffering from drought.

Most of the experiments described in a later section have been carried out on the Series III soils of Mlanje, and the incidence of yellows disease may be judged from the data of those experiments. It will be noted that in Exp. 1 a garden, that had received no treatment other than a recent dressing of a fertiliser probably without influence upon yellows, showed 68 per cent. of bushes moderately or severely diseased. The plots of Exp. 2 showed at the start an average count of 40 per cent. of the theoretical stand diseased and 13 per cent. dead, the deaths having been certainly due in most instances to yellows. These two examples are typical of the conditions in the more seriously affected areas of Mlanje. In Cholo, yellows is less obvious, probably because the tea is mostly of recent planting; but it is present, and its incidence appears to be related to similar factors to those which cause its appearance in Mlanje.

Yellows is often very severe in certain small swampy areas, where water rises to the surface during the rains. Drains cut in these areas carry away a large volume of water. It is uncertain whether the disease is here to be attributed to the leaching of the soil by this flow of water or to the reducing conditions produced by waterlogging.

We have observed a seasonal rise and fall in the incidence of the disease. It is worst in November, at the end of the dry season; thereafter there is usually a gradual improvement up to the end of the rains. This change is most marked in the swampy areas mentioned in the last paragraph. On the Series I soils it may not be obvious. It has been evident in many of our experiments on the red soils, where regular observations have been maintained. For this reason we have endeavoured to make our final records at the end of one or two full years from the start of each experiment. Where this precaution is not observed (*e.g.* Exps. 8, 9, 10) it is important to differentiate between the natural change in the healthiness of experimental bushes and that due to treatment.

Until recent years little use has been made by Nyasaland planters of artificial fertilisers. Where recently they have been applied, they have usually been of the highly concentrated types lacking sulphur, which, as we shall show, is the essential element in the control of this disease.

All of the several types of plants grown from local seed, which form the bulk of the Nyasaland tea, are subject to yellows disease. On the whole, however, they appear to suffer less than plants raised from seed recently imported from Assam and Ceylon. Plants of all ages may suffer; young seedlings in the nursery, young and old transplants, and young and old plants which have grown undisturbed from seed set at stake.

V. FIELD EXPERIMENTS WITH FERTILISERS.

The design of reliable experiments for the study of the effect upon yellows disease of soil treatments holds problems not normally encountered by the field experimentalist. We began this work 2½ years ago unacquainted with any similar study which might have served as a model. Many of the difficulties and the manner of overcoming them have been realised only gradually as the work progressed. In consequence the design of certain of our experiments is open to criticism. In all, however, we have obtained large effects from treatment; we have therefore felt no hesitation in presenting certain results, the significance of which cannot be determined statistically.

The difficulties in experimentation with tea yellows have lain in the lay-out of experiments and in the estimation of their results. The problem may be attacked from two angles: (1) the cure of bushes already diseased, and (2) the protection of new plantings from the disease. For experiments in the former, recourse must be had to old established gardens. Such gardens consist of (a) healthy bushes, (b) bushes in all stages of the disease, and (c) many blank spaces where bushes have died out from the disease. The distribution of the several categories is never approximately

uniform over any considerable area. Consequently these areas afford highly unsatisfactory material for the lay-out of plot experiments. If experiments be performed upon new plantings, it is necessary to choose a site for the experiment where the disease may be expected to appear naturally in untreated plants. The dark soils (Series I) satisfy this condition; but elsewhere a suitable area can be obtained only by clearing land which has borne old diseased tea. But such land will in part have laid fallow (where bushes have died out in the past) and will in part have recently borne either healthy or diseased bushes. Such an area is therefore the opposite of uniform in respect of the factor that produces yellows disease.

The results of experiments must be assessed in terms of a quantity, the incidence of disease, which is not readily subject to exact measurement. A mere record of a bush as diseased or healthy affords a highly incomplete picture, when so many degrees of disease exist. It disregards a change, for example, from a severely diseased to a slightly diseased condition. But such a change may be as important as a change from a mildly diseased to a healthy condition.

Our attempts to overcome these difficulties will be seen in the descriptions of the experiments which follow. We may anticipate by saying that we have found it most satisfactory to work with the single bush as our unit. We obtained no advantage by selecting the bushes in groups and applying a different treatment to each bush in the group, over a simple random distribution. Our methods of estimating the severity of the disease in the individual bush and of the statistical treatment of the results are explained in the Appendix.

Exp. 1. An experiment in the treatment of diseased bushes with a mixture of artificial fertilisers was started, before we began our investigations, by Mr A. E. Shinn, to whom we are indebted for early details and for permission to make observations.

Site. Garden on red soil (Series III), heavily cropped in the past, said to have been chosen in 1927 by Dr Butler as the worst garden on the estate.

Plants. Old bushes of local mixed jat.

Lay-out. Two adjacent blocks. Records made on observation rows next but one to dividing brake.

Treatments, per bush:

<i>Block A.</i>	December, 1927	14 gm. ammonium sulphate.
	March, 1928	17 gm. ammonium sulphate, 8 gm. potassium chloride.
	March, 1929	14 gm. double superphosphate, 14 gm. urea.
	July, 1929	36 gm. "Nitrophoska" complete mixture.
	March, 1930	17 gm. ammonium sulphate.
	October, 1930	17 gm. ammonium sulphate.

Block B. Nothing up to July, 1929.
From July, 1929 onwards as Block A.

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Results. Records made by us from December, 1929:

Date of observation	Block A			Block B		
	Percentage healthy	Percentage chlorotic	Percentage diseased	Percentage healthy	Percentage chlorotic	Percentage diseased
28. xii. 29	88	5	7	32	34	34
20. iii. 30	90	8	2	50	20	30
28. xii. 30	93	6	1	76	11	13
27. iv. 31	99	0.5	0.5	94	3	3
21. xii. 31	98	2	0	83	15	2

The estate records showed that a marked improvement had been visible in Block A as early as 1 month after the first application of ammonium sulphate. By December, 1929, this tea was largely healthy, as the figures demonstrate. At this time the block stood out as a green patch in an area otherwise predominantly yellow. It is clear, therefore, that this experiment had already shown the way to the cure of yellows disease, before we began our investigations. Unfortunately its significance had not been correctly interpreted; the success achieved was attributed to a general effect of fertilisation rather than to a particular effect of one of the materials employed; and it was followed in 1929 by a treatment of the whole estate with a "Nitrophoska" mixture¹. This treatment had little or no effect upon the disease. (The improvement that our records show between December, 1929 and March, 1930 might be due to a delayed action of the Nitrophoska applied in the previous July, or equally to the normal seasonal improvement to be expected during these months.) In the following year the error was recognised, and ammonium sulphate was applied generally over the estate. The effect of this treatment rapidly became obvious in our observation blocks, as well as generally over the estate. Block A made a further slight improvement, so that in December, 1931, 98 per cent. of bushes were healthy. Block B improved greatly; the percentage of healthy bushes rose from 32 in December, 1929, to 83 per cent. in December, 1931, while the percentage of fully diseased bushes fell in the same time from 34 to 2 per cent.

Exp. 2. The first experiment laid out by us was for the purpose of observing the effect upon old diseased bushes of treatment with forms of the three common fertilising agents.

Site. Red soil (Series III) somewhat surface washed.

Plants. Old bushes of local mixed jat.

Lay-out. Four parallel plots, each of four rows. Observation maintained on two middle rows.

¹ This material is reputed to be a mixture of ammonium nitrate, diammonium phosphate and potassium chloride.

Treatments (applied December, 1929), per bush:

- Plot A. Double superphosphate, 42 gm.
 „ B. Double superphosphate + potassium sulphate, 28 gm.
 „ C. Double superphosphate + potassium sulphate + ammonium sulphate, 42 gm.
 „ D. Control—no treatment.

Results. Records of bushes as diseased or “commercially healthy” (*i.e.* scoring 95 per cent. or over, see Appendix). Final results in December, 1931:

	Plot A	Plot B	Plot C	Plot D
Total plants in observation rows	87	83	89	90
<i>Start.</i> Total plants healthy	49	37	50	53
<i>End.</i> Total plants healthy	36	56	77	43

The results of this unreplicated experiment are not subject to statistical analysis. The indications are clear however. The healthiness fell during the two years in the control plot and in that treated with double superphosphate¹. On the other hand, a marked improvement followed treatment with potassium and ammonium sulphates, particularly when the two were combined in Plot C. In this plot almost all the plants had improved greatly, and at a glance it appeared to be entirely healthy.

Exp. 3. In this experiment we studied the effect of a mixed fertiliser in preventing the appearance of yellows in new plantings upon the dark soil of Series I.

Site. Series I soil, newly cleared from bush.

Plants. Nursery stock of local mixed jat.

Lay-out. Sixteen plots of 64 plants each (with marginal rows), grouped in blocks of two plots, one of which, chosen at random, received treatment. Planted 2. ii. 31.

Treatments (applied 6. iii. 31), 33 gm. per bush of a mixed fertiliser, consisting of:

Diammonium phosphate	70 per cent.
Potassium sulphate	15.5 per cent.
Potassium chloride	12 per cent.
Magnesium oxide	2.5 per cent.
Controls	no treatment.

Results. Final records made 29. iii. 32 (*i.e.* 1 year from start):

	Treatments		Standard error	z test
	Fertilised	Unfertilised		
<i>Total stand, % of possible stand</i>	88.0	85.9	±3.54	Not significant
<i>Healthy plants, % of total stand</i>	88.1	36.9	±3.35	> 1 % point

An analysis of the figures from this replicated experiment showed that there was no significant difference between the fertilised and unfertilised plots in the total stand of plants which became established; that is, the failure to realise a full stand is to be attributed to other causes than the

¹ This material, unlike the old superphosphate, appears to contain little or no calcium sulphate.

fertility of the soil. Fertiliser treatment had a highly significant effect in preventing the appearance of yellows disease, the difference in the percentage of healthy plants amounting to more than fifteen times the standard error.

These preliminary experiments demonstrated that tea yellows was a disease which might be largely cured or prevented by treatment with certain fertilisers. The variety of treatments employed allows of no certain conclusion as to the particular material responsible for the effect. In every instance, however, where improvement was noted the treatment included either ammonium or potassium sulphate. In the experiments next described a comparison was made between these and other sulphates and other potash- and nitrogen-containing compounds.

Exp. 4. This experiment was designed to compare the effects on old diseased bushes of potassium sulphate and potassium chloride, at the rate of 42 gm. per bush. The results at the end of a year showed a statistically significant effect from either treatment over no treatment, and an advantage, just significant, of the sulphate over the chloride. The materials used were commercial fertilisers, and a subsequent analysis revealed a sulphate content equivalent to 1.6 per cent. potassium sulphate in the sample of the chloride. The apparent response to potash in any form therefore is under suspicion, although the total dose of sulphate given to each "chloride" bush amounted only to 0.65 gm. of potassium sulphate.

Exp. 5. This small experiment compared ammonium sulphate with sodium nitrate. The results are not subject to statistical analysis. The effect was, however, very striking. The disease increased greatly in bushes treated with sodium nitrate, while a large proportion of those receiving ammonium sulphate recovered completely.

Exp. 6. Ammonium sulphate was compared with other forms of nitrogenous fertilisers (including whale-meat meal as a source of organic nitrogen) in a plot experiment with new plantings on an area in an old tea garden from which the old bushes, largely diseased, had been removed.

Site. Old garden on red soil (Series III), which had borne many severely diseased bushes.

Plants. Nursery stock of local mixed jat.

Lay-out. Four randomised blocks, each of five plots. Each plot of 56 plants with double marginal rows. Planted 9-11. ii. 31.

Treatments (applied 28. ii. 31, whale-meat meal on 28. iv. 31), per bush:

Ammonium sulphate	40 gm.
Urea	18 gm.
Sodium nitrate	53 gm.
Whale-meat meal	60 gm.
Controls	no treatment.

Results. Final records on 3. ii. 32 (*i.e.* approximately 1 year from start).

	Treatments					Standard error	z test
	Ammonium sulphate	Urea	Sodium nitrate	Whale-meal meal	Control		
Total stand, ¹ % of possible stand	88.8	84.8	79.0	83.9	76.3	±4.50	Not significant > 1 % point
Healthy stand, % of total stand	62.2	15.8	21.6	30.3	27.1	±7.64	

In this experiment, as in Exp. 3, the total stand of plants obtained showed no significant difference between the several treatments. The factors, other than yellows, that caused the death of many young plants, were therefore unaffected by the fertilisers used. The proportion of healthy plants in the plots receiving ammonium sulphate was significantly greater than in any other plots. Other treatments showed no advantage over the control plots. At the end of the year just over 60 per cent. of the plants receiving ammonium sulphate were healthy. This proportion is not high, but it may be remarked that great difficulty has usually been encountered by planters in establishing a healthy stand of young tea on old tea lands, and this experiment was sited in an exceptionally bad yellows area.

Exp. 7. Here we compared ammonium sulphate with certain other sulphates and with certain nitrogenous fertilisers not containing sulphur.

Site. Two old gardens on red soil (Series III).

Plants. Old diseased bushes, local mixed jat; mean percentage healthiness at start, 76 per cent. (see Appendix).

Lay-out. Thirty groups each of six diseased bushes chosen, and each of six treatments applied to one bush chosen at random in the group.

Treatments (applied 25. i. 31-6. ii. 31), per bush:

Ammonium sulphate	40 gm.
Magnesium sulphate	42 gm.
Sodium sulphate	53 gm.
Sodium nitrate	53 gm.
Urea	18 gm.
Controls	no treatment.

Results. Final records on 22. i. 32. Estimated as described in Appendix.

	Treatments					Standard error
	Ammonium sulphate	Magnesium sulphate	Sodium sulphate	Sodium nitrate	Urea Control	
Mean percentage increase in healthiness	+89	+81	+82	-29	+2 +1	±11.4

z test—for treatments, > 1 per cent. point;
for sulphate *v.* no sulphate, > 1 per cent. point;
for other comparisons, not significant;
for arrangement in groups, not significant.

¹ These figures include those plants that became established and later died of yellows; that is, the deficit on the possible stand consists of plants that died of other causes than yellows disease.

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The analysis of the results of this experiment showed that there was a large significant response to sulphate in any of the three forms; that there was no differential response between the three forms of sulphate; and that the nitrogenous fertilisers that contained no sulphur were without effect. The apparent depressing effect of sodium nitrate, though large, is not significant. It will be noted that the arrangement of bushes in blocks afforded no advantage over a random distribution through the garden.

Exp. 8. An experiment on somewhat similar lines was laid out upon the dark soil (Series I). The bushes were of the Kyang variety, 3 years old from seed planted in the field at stake. The lay-out and execution of this experiment were faulty, and we do not quote the results in detail. Nevertheless the results confirmed our previous experiments. Bushes treated with the sulphates of ammonium, potassium, sodium and magnesium all improved considerably and about equally. Sodium nitrate, potassium chloride and whale-meat meal were without effect as compared with the controls. A considerable improvement resulted from treatment with a material reputed to be double superphosphate; a subsequent analysis of this material however revealed a large sulphate content ($\text{SO}_4 = 12.5$ per cent.).

Our three final experiments tested the effect of dressings of elemental sulphur, in comparison with combined sulphur and other materials.

Exp. 8.

Site. Series I soil, recently planted on newly cleared land.

Plants. Young bushes of local mixed jat. Mean percentage of plants healthy at start, 59 per cent.

Lay-out. Duplicate strip plots, each of 50 plants, with marginal rows.

Treatments (applied 7. i. 32), per bush:

Sulphur	5 gm.
Sulphur	10 gm.
Potassium sulphate	28 gm.
Control	no treatment.

Results. Final records taken on 21. iv. 32 (i.e. $3\frac{1}{2}$ months from start).

	Treatments				Standard error
	Sulphur 5 gm.	Sulphur 10 gm.	Potassium sulphate	Control	
Mean percentage increase of healthiness of plots	+42	+49	+59	-25	± 9.6

z test—for treatments, >5 per cent. point;
for sulphur *v.* potassium sulphate—not significant.

Exp. 9.

Site. Red soil (Series III), badly diseased garden.

Plants. Old diseased bushes, local mixed jat; mean percentage healthiness at start, 76 per cent.

Lay-out. Each treatment applied to each of 20 diseased bushes, distributed at random over garden.

Treatments (applied 12. 1. 32), per bush:

Sulphur	5 gm.
Sulphur	10 gm.
"Nitrophoska" mixture	28 gm.
"Nitrophoska"	28 gm. per bush and sodium sulphate 28 gm.
Control	no treatment.

Results. On 19. iv. 32 (i.e. 3 months from start).

	Treatments					Standard error
	Sulphur 5 gm.	Sulphur 10 gm.	Nitrophoska	sod. sulphate	Control	
Mean percentage increase in healthiness of bushes	+66	+72	+25	+59	+40	±7.7

z test—for treatments, >1 per cent. point;
for sulphur *v.* no sulphur, >1 per cent. point;
for Nitrophoska alone *v.* control, not significant.

Exp. 10.

Site. Red Soil (Series III), old tea much neglected in past and overgrown with weeds up to time of treatment.

Plants. Local mixed jet; mean percentage healthiness at start, 63 per cent.

Lay-out. Nine groups, each of eight diseased bushes. Within the group, each bush received a different treatment, selected at random.

Treatments (applied 31. x. 31). Ground sulphur at rates of 32, 16, 8, 4, 2, 1, 0.5 gm. per bush and untreated controls.

Results. On 28. iv. 32 (i.e. 6 months from start).

	Treatments. Sulphur (gm.)							Standard error
	32	16	8	4	2	1	0.5	
Mean percentage increase in healthiness of bushes	+93	+98	+91	+94	+83	+48	+87	+56 ±10.8

z test—for treatments, >1 per cent. point;
for arrangement in groups, not significant.

In each of the three preceding experiments, one on the dark soil and two on the red, a significant response followed applications of uncombined sulphur. The response was evident within a few months. The control plants in Exp. 8, as was to be expected with new plantings upon the dark soil, degenerated considerably during the 3½ months of the experiment. Those treated with sulphur on the other hand—and equally those treated with potassium sulphate—improved in healthiness in this period to the extent of about half the possible improvement. Potassium sulphate was not significantly more effective than elemental sulphur. Exp. 10 showed a considerable improvement in the control bushes, due doubtless in part to the cultivation following a period of neglect and in part to the normal seasonal improvement which, as we have already noticed, may be expected during the summer months in which our observations fell. The

improvement of the bushes treated with sulphur was however significantly greater than that of the controls, and with the larger applications the bushes had become almost entirely healthy. The result of the treatment with 1 gm. of sulphur is anomalous, and casts doubt upon the reliability of the results of small applications. Nevertheless it may be deduced that under the conditions of this experiment an application of more than 4 gm. of sulphur is effective. It will be noted that in Exp. 9, Nitrophoska appeared to have a depressing effect, although not significant on the figures available.

The evidence provided by the foregoing experiments points consistently to sulphur as the element concerned in the amelioration of the yellows condition in tea. In all instances an improvement, generally statistically significant, has followed the application of a sulphur-containing material to the soil. On the other hand treatment with a wide variety of other materials, containing little or no sulphur, produced no effect on the disease or gave indications of increasing it. The only apparent exception was the response to potassium chloride in one experiment, and here the small admixture of potassium sulphate, found to be present in the sample used, makes doubtful the interpretation of this result.

The improvement obtained by sulphur treatment of diseased bushes has sometimes amounted to a complete cure. More often, however, our data show a significant improvement not amounting to a complete cure. It must be remembered, however, that, by expressing our results in terms of the mean (which allowed us to estimate their significance), we could only get the maximum possible improvement if every treated bush made a complete recovery. In fact, a large number of the treated bushes recovered completely (*e.g.* Plate IV, fig. 8). The improvement observed in others indicated clearly that in time they would be completely healthy. Our experiments have mostly run for a year or less. In the one experiment (No. 1) in which 4 years elapsed since the first treatment, the treated bushes were, for practical purposes, entirely healthy.

For the most part our experiments were performed on bushes in only a moderately advanced stage of the disease. We considered that in the late stages of the disease secondary influences might prevent a recovery even when the primary cause of the disease was removed. Nevertheless we have frequently been successful by treatment with sulphates in bringing back to health bushes that appeared to be on the point of death (*i.e.* in the condition illustrated in Plate IV, figs. 6 and 7).

These experiments in the treatment of the soil leave in doubt the part played by the sulphur. Either the sulphur supplied enters directly into

the nutrition of the tea bush, or it exerts some indirect chemical or biological effect upon the soil. In the following sections we shall give the evidence which leads us to believe that the function of the sulphur is nutritional.

VI. EXPERIMENTS IN WATER CULTURE.

In the following experiments we endeavoured to reproduce the symptoms of yellows disease in tea seedlings grown in solutions of known composition. The methods followed conventional lines (see Brenchley(1)). Tea seedlings were raised in washed river sand, watered with distilled water, from seeds of the Assam-type plants growing in Amani. When a shoot of a few centimetres had developed, the seedling, after washing in distilled water, was set in the culture bottle with the cotyledons below the cork, so that the shoot emerged through a hole in the cork. The bottles were of 2 litre capacity.

The solutions employed were based upon Brenchley's formula (1), p. 12). "A.R." chemicals (British Drug Houses, Ltd.) were used throughout. The water was distilled in a tinned copper still and was proved to be free from copper. The full nutrient had the following composition:

Full nutrient potassium nitrate, 0.5 gm.; sodium nitrate, 0.5 gm.; magnesium sulphate, 0.25 gm.; potassium dihydrogen phosphate, 0.25 gm.; calcium chloride, 0.25 gm.; ferric chloride, 0.04 gm.; water, to 1 litre.

The remaining solutions were prepared by substituting, as required, chloride for sulphate, sulphate or chloride for nitrate, sulphate for phosphate, sodium for potassium, potassium for magnesium, and calcium nitrate for calcium chloride. Air was bubbled through each bottle for a short period every third day. To one half of the bottles in every series we added boron and manganese in the proportions of 0.0005 gm. boric acid and 0.001 gm. potassium permanganate (or 0.0015 gm. manganese sulphate) per litre. We observed, however, no consistent advantage from these additions. The initial pH of all the solutions was 3.3-3.4 (determined by the quinhydrone electrode method). This high acidity was shown by Gadd(8) to be favourable for tea in water culture; and our preliminary trials had shown a considerably less acid solution to be unfavourable. During the course of the experiments however a gradual spontaneous change to a less acid condition (pH 3.5-4.1) occurred.

Our first experiment compared the qualitative effect of withholding the following elements: sulphur, nitrogen, potassium, phosphorus, magnesium, chlorine. Each series consisted of four seedlings growing in

separate bottles. The plants in all series made early satisfactory growth of shoot and root. Those receiving the full nutrient (except one—see below) continued in healthy normal growth to the conclusion of the experiment, except that a little scorching occurred upon the older leaves (Plate V, fig. 11). Observation was maintained for 6 months.

The series of plants which received all nutrients except sulphur rapidly developed symptoms typical of yellows disease (Plate V, fig. 12). At the end of 2 months one plant had produced mottled leaves, with the lamina pale and the veins dark, and later it produced only minute yellow uprolled leaves, which were quickly shed (Plate VI, fig. 13). The remaining three plants did not show the mottled phase, but passed at once to the production of small uprolled yellow leaves, which scorched on the margins and soon fell (Plate VI, fig. 14). Meanwhile in all these plants the growth of the stem was much reduced, all internodes being very short. After 14 weeks, one plant, which had previously reached the chlorotic small leaf condition, received potassium sulphate at the rate of 0.25 gm. per litre. The one unshed yellow leaf, which was at this time half-scorched, became green in 10 days (Plate VI, fig. 15), and later the shoot grew out into healthy green leaves of normal texture, but somewhat undersized (Plate VI, fig. 16).

The plants in the remaining series showed various abnormalities of growth, but none resembling yellows disease. Those lacking nitrogen bore pale green leaves, but of normal size and texture, and growth soon ceased. The leaves of the plants lacking potassium, phosphorus, chlorine and magnesium were normal in colour and texture, but (except in the phosphorus series) scorching was more severe than in the full nutrient series and in all the growth was inferior. The plants lacking magnesium shed all leaves rapidly, thus superficially resembling those affected with yellows disease; but at no stage did they produce the uprolled yellow leaves typical of this disease.

Minor differences were evident in the form of the root systems between the several series, but in all they developed strongly and remained healthy and functional, except that the root of one plant receiving full nutrient was killed by a fungus, and some of the lateral rootlets of the plants lacking nitrogen and chlorine died back. None of these plants at any stage showed symptoms resembling those of yellows disease.

A second experiment exactly repeated the first in respect of the series receiving full nutrient and that lacking sulphur. The results were almost identical. All the plants deprived of sulphur showed the mottling of the young leaves after 2 months, and later produced only the small yellow

leaves, scorched on the margins, typical of yellows disease. A parallel series, deprived of all nutrients (except that hydrochloric acid was added to the distilled water to bring the initial pH to 3.3), made an early growth similar to that shown by the series lacking nitrogen. Later, however, all growth of root and shoot ceased. The leaves remained pale green and small, but of normal shape and texture. The roots died back without producing lateral rootlets. The condition of these plants did not resemble yellows disease.

In a third experiment the seedlings were set in a solution lacking sulphur (of the same composition as previously), to which had been added sodium sulphate at rates of 0, 0.00027, 0.0027, 0.027 and 0.27 gm. per litre. Typical yellows symptoms appeared in all plants receiving no sulphate and in those receiving 0.00027 and 0.0027 gm. of the sulphate per litre. The symptoms were developed only mildly in the last. The plants that received 0.027 and 0.27 gm. of the sulphate per litre made healthy growth.

VII. EXPERIMENTS IN ABSORPTION THROUGH CUT BRANCHES.

The following experiments, carried out upon bushes in the field in Nyasaland, studied the influence on yellows disease of different salts when introduced directly into individual branches, which throughout the experiment remained attached to the parent bush. This introduction was effected by cutting a small side shoot under water and immersing its cut end in a solution of the required salt. The solution taken up was made good daily and the ends of the immersed shoots were cut back to unchoked wood every fourth day.

Preliminary trials showed that as a result of the absorption of a 0.5 per cent. solution of the sulphates of either magnesium, potassium or sodium, the leaves borne by the branch, beyond the cut shoot, rapidly developed a normal green colour and thereafter healthy growth followed. The absorption of salts other than sulphates from solutions of 0.5 per cent. concentration had no effect upon the disease, but scorching of the leaves frequently occurred with nitrates and chlorides.

An experiment was carried out in the treatment of branches by this method with solutions of four salts of potassium—the sulphate at 0.36 per cent., nitrate at 0.28 per cent., chloride at 0.21 per cent., and dihydrogen phosphate at 0.36 per cent. The concentration of the solutions was chosen to be approximately isosmotic. Fifteen bushes were selected in an advanced stage of the disease, and two branches on each bush were treated, one with the sulphate and the other with one of the remaining

salts. Thus fifteen branches were treated with the sulphate and five branches with each of the other salts. A greening of the leaves of some of the sulphate-treated branches was visible within 3 days. At the end of 14 days the condition of the treated branches was as follows: of the sulphate-treated branches, eight showed marked greening and the beginning of new healthy growth, five showed slight greening and two showed no effect; of the branches treated with the nitrate, chloride and phosphate, none showed any change. The photograph in Plate V, fig. 10 illustrates the typical effect after 20 days. Here both branches, borne upon the one bush, were at the start in the condition of the upper branch. This branch had absorbed from the test-tube a solution of potassium chloride and had remained unchanged. The small leaves of the lower branch, which had absorbed potassium sulphate, had become green, and the terminal bud had grown out into a flush of large healthy glossy green leaves.

VIII. EXPERIMENTS IN THE UNILATERAL TREATMENT OF BUSHES.

A difficulty of the deficiency hypothesis of the causation of tea yellows has been to explain the unevenness of the distribution of the disease over a single bush. We have already mentioned that one may occasionally find a bush one half of which is severely diseased while the other half is healthy. It would be expected that a soil deficiency would show its effect uniformly over the whole bush.

We have carried out certain experiments which throw light upon this phenomenon. Nearly uniformly diseased bushes were treated by adding potassium sulphate to the soil on one side only. Where the bush showed originally any difference, the fertiliser was applied on the healthier side. After 2 months, a definite improvement was visible upon the sides of the bushes that had received the treatment, while the opposite sides had not changed. A similar result was obtained, when all the lateral roots on the unfertilised side of the bush were cut, down to a depth of 2 ft. If, however, the lateral roots on the unfertilised side *and* the taproots were cut, those bushes which survived this treatment made a uniform improvement throughout the whole bush. Control bushes that received no fertiliser showed no unilateral effect with the lateral roots alone cut and no general improvement when laterals and taproot were cut.

We conclude from these experiments that there is a tendency in the tea bush for each root to supply only those branches immediately above it. The sulphate, absorbed plentifully by the lateral roots on one side of the bush, passed only into the branches on that side of the bush. Even if

all the lateral roots on the opposite side of the bush were cut away, the branches on that side appeared to be maintained by the water absorbed by the taproots. Only when all the roots other than those receiving the fertiliser were cut away did the absorbed sulphate pass to all branches of the bush.

It is thus not difficult to perceive how in the field an uneven distribution of the disease within a single bush may come about. If certain roots of a bush extend into an area of soil denuded of its sulphates, yellows disease may be expected to appear in the branches subtended by those roots, while the remainder of the bush remains healthy. It is significant that, in the unilaterally affected bushes that are found on the sides of drains, the diseased half usually faces towards the side where leaching and erosion have been most severe.

IX. EXPERIMENTS WITH TOBACCO.

Experiments confirming our conclusion that Nyasaland tea soils are deficient in available sulphur have been carried out with tobacco. We chose this plant because of its rapid strong growth, and because the symptoms of deficiency diseases in it have been frequently studied.

Tobacco plants, of the variety Connecticut Havana, were set in 6-in. clay pots containing top soil from a Nyasaland tea garden where yellows disease was prevalent. Four series of plants received the following treatments: distilled water, a full nutrient solution (of the same constitution as that used for the water culture experiments), a full nutrient lacking only sulphur, and a full nutrient lacking magnesium (since at the time we suspected magnesium deficiency to be the cause of yellows in tea). The plants received no water other than the nutrient solutions; at each watering the solution was added until the soil was saturated and the liquid flowed from the drain-hole of the pot. The conditions were very severe, since a comparatively small volume of soil was repeatedly heavily leached. They were likely therefore to reveal an actual or potential shortage of sulphur or magnesium in the soil sample used. This method was employed successfully by Garner *et al.*⁽¹⁰⁾ in studies of magnesium-deficient soils.

The plants receiving only distilled water made little growth and remained stunted and yellow. Under the conditions of the experiment the plants apparently suffered from a general starvation and showed no characteristic symptoms. The plants receiving full nutrient, on the other hand, grew into large plants, of a healthy green colour (cress green,

29" k)¹, and flowered normally. Those receiving no sulphur grew equally well for the first 45 days. Thereafter a diffuse chlorosis began to appear, following the larger veins of the young leaves, and producing a characteristic mottled effect. The older leaves became progressively paler, while the chlorosis was more pronounced on the new leaves formed. Eventually the yellow-green colour was general throughout the plant and the early mottling was lost. At the end of 97 days the plants were only slightly smaller than the series receiving full nutrient, but their colour, chrysolite green (27" b), was strikingly different. Their leaves tended to be shorter and wider, but were not detectably different in texture. Flowering was slightly delayed. After 84 days, one plant received an addition of sodium sulphate to the soil. A slight greening was visible in 3 days. After 5 days the youngest leaves were a nearly normal green. By the thirteenth day this plant was a full healthy green in all but its oldest leaves, and was only just detectably paler than the plants which had received full nutrition throughout their life. The plants which received no added magnesium were indistinguishable throughout their growth from those receiving full nutrients. The characteristic symptoms of magnesium deficiency, "Sand-drown" disease (10), were absent.

Similar results were obtained in a second experiment. The plants from which sulphur was withheld entirely became severely chlorotic. On the other hand a series which received 5 gm. of calcium sulphate, incorporated with the soil at the start, and thereafter only the nutrient lacking sulphate, was as healthy as the series which received the complete nutrient solution throughout the experiment. The addition of 1 gm. of calcium sulphate to the soil of a plant which had reached the chlorotic condition caused it to become fully green within 7 days. The addition of 1 gm. of ground sulphur was equally effective, but recovery was slower.

Plot experiments with tobacco on old tea land in Nyasaland, cleared of diseased tea bushes, gave similar results. The Mlanje conditions are ill-suited to tobacco growing, and all plants were poor. Nevertheless there was a clear differential effect between plots receiving sulphates and those receiving other fertilisers. Various combinations of sodium nitrate, double superphosphate and "Nitrophoska" mixture with either potassium chloride or potassium sulphate were compared. The characteristic pronounced mottling was visible in the majority of the plants in the plots which received no sulphate, and a few reached the stage of uniform chlorosis. The plants which received potassium sulphate were all healthy

¹ Colour nomenclature according to Ridgway, *Colour Standards and Nomenclature*, Washington, D.C., 1912.

or showed a faint mottle only. The growth in the sulphate plots was notably stronger than in the other plots.

X. THE PART PLAYED BY *RHIZOCTONIA BATATICOLO*.

In 1930 Dr W. Small drew our attention to the presence of the sclerotia of *Rhizoctonia bataticola* in the roots of bushes which had died of yellows disease. We have fully confirmed his observations. Of thirty-five old bushes which had died of yellows on one estate, all bore sclerotia in their roots; of twenty-five similar dead bushes on another estate twenty-three definitely contained sclerotia. Many similar examinations of old and young bushes, which had died of yellows, revealed *R. bataticola* in the roots of a considerable proportion of the plants. In many plants the fungus had advanced into the larger roots.

We have frequently found *R. bataticola* in the small rootlets of plants which are in an advanced stage of yellows, although not dying of the disease. Thus of seventy-eight diseased but still living "supplies," eighteen showed definite evidence of *R. bataticola* in the fine rootlets. These eighteen plants were noted as being generally in a more advanced stage of the disease than the remaining sixty. A similar situation was found in 145 diseased seedlings removed from a nursery bed, of which half showed sclerotia in the finer rootlets. The fungus was more readily found in the plants in the later stages of the disease; nevertheless 28 per cent. of the plants in the early chlorotic phase of the disease bore sclerotia.

If the difficulty of the complete removal of the rootlets of a plant and of the certain recognition of this fungus in the field be held in mind, the association of *R. bataticola* with yellows disease will be seen to be close. The observational evidence appears to support the hypothesis that yellows is a root disease caused by *R. bataticola*. This hypothesis had been advanced by Small(25).

Experimental evidence, however, has failed to show that *R. bataticola* may initiate the condition of yellows disease in plants suffering from no nutritional shortage, or to show that the fungus can prevent the recovery of moderately diseased plants when the hypothetical nutritional shortage is made good.

For inoculation experiments we have used a pure culture of *R. bataticola* isolated from a root of a yellows-diseased bush. The sclerotial measurements—an average diameter of 187μ based on a total of fifty counts—place this culture in Haig's strain B(13). We have not isolated Haig's other strains from Nyasaland tea.

Pot experiments were carried out in the following manner. A sample of Nyasaland top soil was filled into twelve clay pots and autoclaved for 3 hours. Soil taken from the centre of the bulk and inoculated on to agar gave no growth of any organism. It should be noted that heat treatment of this soil appears to increase the availability of nutrient constituents, and that tea seedlings normally make strong healthy growth in it. Nevertheless one half of the pots received doses of a complete nutrient solution after the seeds had germinated. Six of the pots were inoculated with *R. bataticola* by incorporating two young agar "slant" cultures with the soil in each. Tea seeds, surface sterilised, were set in all pots. After the seeds had germinated, the soil in the originally inoculated pots was reinoculated by placing agar bearing the fungus in a hole near to the seedling. The pots were watered as needed with the nutrient solution or freshly distilled—but not sterilised—water. No difference in the top growth was evident in inoculated and control seedlings, which were all uniformly healthy. After 5 months the roots were carefully washed out. The root systems of all plants were entirely healthy, with the sole exception of one single root tip in one inoculated plant. This root tip was dead, but contained no sclerotia of *R. bataticola*.

Inoculations were made in the field into roots of established tea bushes. The roots were exposed by the removal of the soil, surface sterilised, and a pure culture of *R. bataticola* was placed (a) into V-cuts in roots 10–20 mm. diameter, and (b) on to the cut ends of roots 5–10 mm. diameter. A pad of moist cotton-wool covered the inoculation cut. Both healthy and yellows-diseased bushes received the inoculation. Half of the bushes were heavily pruned immediately after the inoculation and half were left unpruned. Three months later the roots were cut off for examination. *R. bataticola* had failed in every instance to enter the larger roots through the V-cuts. It had also entirely failed to invade the smaller roots of healthy or mildly diseased bushes. Only in the small roots of severely diseased bushes had it made headway. In all of twenty-one inoculations of severely diseased bushes a penetration of the fungus was noted, varying from 7 mm. to between 100 and 300 mm. (in nine of the twenty-one roots). The pruned bushes were not more severely attacked than the unpruned. Out of twenty-one control roots of severely diseased bushes, cut but not inoculated, two were invaded to a distance of 150 mm.

The evidence already given proves that sulphur treatment is effective in curing yellows disease. Our root studies demonstrated that a diseased bush frequently bears roots invaded by *R. bataticola*. It is certain therefore that many of the treated bushes recovered in spite of an established

invasion by this fungus. We can supplement this evidence, however, by certain direct experimental evidence. Eighteen seedlings, showing the symptoms of yellows disease, were removed from a nursery bed and their roots washed out to permit of an examination for *R. bataticola*. Sclerotia were seen to be present in rootlets of all the seedlings. The seedlings were then set in a bed of good soil fertilised with ammonium sulphate and a commercial general mixture. Nine of the plants failed to survive the severe conditions of transplanting, but the remaining nine grew healthily and 5 months later were free from all symptoms of yellows disease. Five more plants died during the following dry season; although *R. bataticola* may perhaps have been responsible for the death of these plants, it should be noted that they died without showing the characteristic progressive degeneration of yellows disease. The remaining four plants at the end of a year were entirely healthy, and an examination of their roots revealed no sclerotia of *R. bataticola*.

The nursery, whence the diseased seedlings had been obtained, was used for a fertiliser experiment. All healthy plants were removed, leaving only those in an advanced stage of yellows disease. We had found that 50 per cent. of the diseased plants in the neighbouring bed bore sclerotia in their roots, and it is fair to assume that the plants in the treated bed were equally infected. This bed was divided into four plots containing about twenty seedlings each. Two plots received no treatment and two received dressings of ammonium sulphate. At the end of 9 months the condition of the bed was as follows: in the untreated plots, 37 per cent. were dead, 41 per cent. were still diseased and 22 per cent. were healthy¹; in the treated plots, 22 per cent. were dead, 6 per cent. were diseased and 72 per cent. were healthy. Thus a previous invasion of the rootlets by *R. bataticola* had not prevented the recovery of a large proportion of the plants which received fertiliser treatment. *R. bataticola* could not be found in the roots of the plants that had recovered.

Our evidence, so far as it goes, is therefore negative. It offers obvious loopholes for criticism by the exponent of a *R. bataticola* hypothesis of the causation of yellows disease. But negative evidence is always open to criticism. We have, however, positive evidence (in our water-culture experiments) that yellows disease may develop under conditions of sulphur deficiency in the absence of *R. bataticola*. We have positive evidence that the presence of *R. bataticola* within the tissues of a plant

¹ The seedlings were somewhat crowded in the nursery bed before the thinning preparatory to this experiment. The recovery of some of the seedlings in the untreated plots was probably due to the lessening of competition.

does not prevent a recovery from yellows disease, if sulphur be supplied. We do not deny the possibility that *R. bataticola* may under some circumstances produce a fatal root disease of tea, a point which is outside the scope of our investigation. We do not doubt that *R. bataticola* plays a part in accelerating the death of plants weakened by sulphur deficiency, and may prevent the recovery of an extremely weakened plant. But we maintain that the characteristic symptoms of yellows disease are those of sulphur deficiency alone and not of *R. bataticola* root disease.

XI. CONTROL.

Our experiments have demonstrated that the addition of sulphur in some form to the soil will cure yellows disease or prevent its appearance. Considerable evidence has already accumulated to show that in field practice such treatment is as rapidly successful as it was in our experiments. The only instance encountered where a striking change did not follow the treatment was on a severely eroded area of laterite (Series II), where soil texture was poor and the general fertility, as judged by weed growth, very low. Even here a definite improvement was noted following treatment with a mixed fertiliser containing a sulphate.

The exact form in which sulphur is applied to the soil for the control of yellows disease appears to be unimportant. Whether it should be applied as the sulphates of ammonium or potassium alone or in a general mixture, or as crude sulphur, will be decided by other considerations. The simple cure of yellows can be attained most cheaply by dressings of sulphur; we have shown that even so small an amount as 4 gm. per bush (*i.e.* 24 lb. per acre) will effect at least a temporary cure, at a trivial cost. The ultimate solution of the problem of the nutrition of Nyasaland tea is likely to be found, however, in the regular application of a fertiliser mixture, chosen primarily according to the needs of the soil for nitrogen, potash and phosphorus, but so compounded as to provide sufficient sulphur to maintain the plants free from yellows disease.

The possibility that, by treatment of the soil in some manner other than by the addition of sulphur, the sulphur already contained in the soil might be rendered available to the tea plant is largely a matter of theoretical interest. Possibly it might prove to be of practical importance; but meanwhile the cure by sulphur treatment of the soil is both easy and cheap.

XII. DISCUSSION.

We have presented evidence which shows that the yellows disease of tea may be prevented or cured by the addition to the soil of a substance containing sulphur. We conclude that the reaction of this substance with constituents of the soil is not a necessary condition of the cure of the disease; for if a sulphate be introduced directly into the diseased bush (as in our branch absorption experiments) recovery follows. Finally we have produced the symptoms of yellows disease in plants grown in water culture by withholding sulphur, and by withholding this element alone. We believe that this evidence admits of one conclusion only, that yellows disease is the result of a deficit of sulphur in the plant, relative to other inorganic constituents. We make this qualification, because it appears from our water culture and tobacco pot experiments that characteristic symptoms are not produced under conditions of general shortage of all nutrients.

The supply of sulphur in a suitable form to a yellows-diseased plant has resulted in a noticeably rapid recovery, made evident both by the greening of leaves previously yellow and later by a healthy growth of new leaves. We conclude that the chlorotic leaf of the sulphur-starved plant retains its chlorophyll-forming mechanism dormant but intact and capable of functioning as soon as sulphur be supplied. This was well shown by the tea in the branch absorption experiments and by the tobacco in pot experiments. On the other hand the individual chlorotic leaf of the diseased tea bush, which has the structure of an immature leaf, has nevertheless lost the power of further growth. A supply of sulphur causes the small chlorotic leaves to turn green, but they make no further growth.

The strong healthy flush put out by a previously diseased bush after treatment with sulphur might be held to indicate that Nyasaland soils are well supplied with all the other nutrients required by tea. But it must be remembered that during the preceding period the bush was making slow growth limited by the uptake of sulphur. It is likely to have built up ample supplies of other mineral requirements which are at once available if sulphur be supplied. Consequently the early healthy growth which immediately follows the first application of sulphur does not necessarily imply that the supplies of plant nutrients in the soil are sufficient to support a continued strong growth indefinitely, if the sulphur shortage alone be made good.

We are unable to offer any clear explanation of the acute sulphur shortage in Nyasaland tea soils. It may, however, be profitable to

consider some possible causes. In general the supply of available sulphur compounds to the roots of a plant is determined by two opposing sets of processes active in the soil: the processes which release soluble sulphur compounds from the insoluble soil constituents, and the processes leading to the removal of these soluble compounds. In a normal soil (receiving no sulphur from external sources)¹ the release of soluble compounds must be supposed to keep pace with their removal. In Nyasaland soils we must suppose either that the processes leading to the release of sulphur compounds are exceptionally inactive, or that the processes of removal, which compete with the tea roots, are exceptionally active.

The Mlanje tea soils have been formed mainly from metamorphic rocks⁽²⁾, which we should expect to contain sulphides of iron (although we have no definite evidence that this is so). We have seen that analyses of these soils reveal a total sulphur content well within the range encountered in "normal" soils. Sulphides of iron are known to oxidise under weathering and to release their sulphur as sulphuric acid. We may infer both from their present freely draining properties and from their lateritic character, that in Mlanje soils in general (apart from swamp soils) oxidising conditions are and have been active. There is therefore no *a priori* reason to lead us to suspect a failure of the agencies which bring soluble sulphates into the soil solution.

The processes of removal, apart from absorption by the roots of the tea, are leaching by percolating water and absorption by competing organisms. The one feature common to the whole tea area is a highly pervious soil receiving a heavy rainfall; that is, conditions under which leaching is likely to be severe. Furthermore the soils formed under the highest rainfall are the ones that show the earliest sulphur shortage. It is tempting therefore to regard leaching as the critical process in producing sulphur starvation of the tea. But this explanation is difficult to reconcile with the observation that yellows disease decreases in severity naturally during the course of the months when the rainfall is heaviest. (These months, however, are the hottest, when the agencies releasing sulphur may be expected to be most active.) Furthermore, in the soils of the highest rainfall belt, the tea that survives the early yellows phase usually recovers in later years. It may be that the roots have then penetrated to a deep layer of soil not denuded of its sulphates by leaching. Again, it is possible that the known large organic content of these high

¹ In a highly industrialised region large quantities of combined sulphur, liberated into the air in the burning of coal, etc., are carried to the soil by rain. Nyasaland soils cannot receive more than an insignificant quantity of sulphur from this source.

rainfall soils in the virgin state encourages a microflora which at first monopolises the available sulphur at the expense of the tea. On the other hand, in the red soils, sulphur starvation becomes evident only after the soil has been denuded of much organic matter by exposure and erosion. These two opposing observations do not necessarily invalidate an explanation based on organic content of the soil, for it may be that only under an optimum organic content is the sulphur of the soil retained from leaching by percolating water and yet released in sufficient quantities to maintain the growth of tea. But an explanation of sulphur starvation as a function of organic content alone is insufficient, for otherwise sulphur starvation would be a world-wide phenomenon in agriculture. We are still driven to seek an explanation of the sulphur shortage in some peculiar feature of the soil types with which we are dealing.

The Mlanje soils owe their present character to the parent rocks and to the climatic formative processes which have been operative upon them. We have found no reason to attribute the sulphur starvation of tea growing on these soils to any peculiarity of the parent rocks. If we turn for an explanation to the formative processes we must recognise that similar processes have been operative in many other parts of the tropics. We should thus be led to infer that the end-product—the soil—would show similar properties, wherever the processes have been approximately similar. That is, we should expect yellows disease to occur generally in tea grown upon lateritic soils (as defined by Hornby). There are indications that we are correct in this expectation. In Nyasaland soils the sulphur shortage has become so abundantly evident because in the past, owing to the heavy cost of transport to this remote country, the use of artificial fertilisers has been neglected or has been confined to the concentrated types, which usually carry little or no sulphur. In older established tea-growing regions the use of fertilisers has probably been general for many years, and it is almost inevitable that the fertilisers used must have been sulphur carriers. Consequently a potential sulphur shortage in the soils would be largely masked. Nevertheless there is evidence that a disease, closely resembling yellows, is prevalent in tea in the East. This disease has generally been attributed to an attack on the roots by the fungus *Botryodiplodia theobromae* Pat. Butler, who first described it in 1902 in Assam (27), p. 414, has drawn attention to its similarity to yellows disease (2). It is noteworthy that Watt and Mann (27) reported that bushes attacked by this disease in Assam were favourably affected by treatment with ferrous sulphate. In Ceylon, Gadd (9) has recognised that several different conditions have been grouped together as "*Diplodia* disease."

The description, which he has sent us, of the condition now known as "witches' broom disease" in Ceylon, agrees closely with yellows disease. Mann⁽¹⁸⁾ has recorded that dressings of sulphur alleviate the infertile condition of old village sites in north-east India. It may be mentioned that workers in Java⁽²⁴⁾ and India⁽³⁾ have obtained beneficial results from sulphur applications to tea soils, but here the investigators have attributed their results to the consequent increase of soil acidity and not to any nutritive effect of sulphur.

In conclusion, we may note that the need of plants in general for sulphur for their normal metabolism is now well recognised. The possibility of a sulphur shortage in agricultural soils has been considered by many investigators (*e.g.* (4), (7)). Large increase of yield has sometimes been obtained from sulphur dressings, *e.g.* in Australia⁽¹²⁾, in India⁽⁵⁾ and especially in the north-western states of America (*e.g.* (20), (22)). Hitherto observations have in the main been confined to the increase in yield, although incidentally some workers have noted a pale colour in plants starved of sulphur^(6, 20, 22). Other workers have produced a chlorosis in plants grown in water culture without sulphur^(11, 19). We believe, however, that our studies provide the first instance where a specific disease of a crop plant, with characteristic symptoms, has been shown to be due to sulphur starvation.

XIII. SUMMARY.

1. The yellows disease of tea, the nature of which has hitherto remained obscure, has caused serious losses to planters in Nyasaland.

2. It is characterised by a progressive degeneration of the bush, in which the leaves become chlorotic, small, uprolled and of stiff texture, and are finally shed. Eventually the bush dies.

3. The disease usually only appears when the soil has been impoverished by erosion or cropping. On certain soil types it may appear in young plantings on newly cleared land.

4. In field experiments the disease was prevented or cured by treatment of the soil (*a*) with mixed fertilisers that included sulphates, (*b*) with the sulphates of ammonium, potassium, sodium or magnesium, or (*c*) with elemental sulphur.

5. Typical symptoms of yellows disease appeared in tea seedlings grown in water culture deprived of sulphur.

6. Individual branches of diseased bushes in the field made normal healthy growth after absorbing through a side shoot a dilute solution of a sulphate.

7. The application of sulphates to certain roots only of a diseased bush caused a recovery only in the branches subtended by those roots.

8. Tobacco plants grown in Nyasaland soil without added sulphur developed a characteristic chlorosis, which was cured by the addition of sulphur.

9. The part played by *Rhizoctonia bataticola* was studied and it was concluded that this fungus was not concerned in the initiation of the disease.

10. This evidence has led us to conclude that tea yellows disease is produced by a deficit of sulphur in the plant in relation to other essential elements.

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XIV. APPENDIX.

Methods of Estimation and Statistical Treatment of the Experimental Results.

(1) *The estimation of healthiness.* In the experiments described two methods of recording have been employed. In the first, bushes were recorded simply as "diseased" or "healthy." A bush was "healthy" only if it showed no trace of the characteristic chlorosis of the disease, except in Exp. 2, where a score of 95 per cent. healthiness (see below) was accepted as constituting a bush "commercially healthy." The second method involved a scoring of the percentage healthiness, in the following manner. In the field a rough plan of the bush was drawn within a circle, sectors being marked to indicate the relative areas of the bush (as seen in plan) that were healthy or diseased. Since tea is pruned so as to form a flat-topped compact bush, it lends itself readily to this method of graphic representation. Four stages of disease were recognised, corresponding to the conditions shown (uniformly) by the bushes in Plate IV.

Stage	Description	Factor
—	Healthy	×4
1st	Leaves chlorotic, full size (Fig. 4)	×3
2nd	Leaves severely chlorotic, small, uprolled (Fig. 5)	×2
3rd	Leaves smaller, mostly shed except at tips of shoots (Fig. 6)	×1
4th	Shoots dying back or dead (Fig. 7)	×0

The areas of the circles corresponding to each category were estimated by eye as a percentage of the whole, and multiplied by the factors shown above. The sum of the products, divided by 4, gave the figure "Percentage healthiness of the bush."

This method is clearly a crude approximation and subject to considerable personal error. All estimates were, however, made by one observer only (R. L.). At least, they record the condition of the bush with more accuracy than a simple eye estimate could attain.

(2) *Statistical treatment of results.* The plot experiments, in which we record the number of bushes healthy after treatment, afford suitable material for an analysis of variance by the methods laid down by Fisher¹. The essence of this process is the application of the z test to the comparisons considered. We have given the calculated value of z in relation to the 1 or 5 per cent. points in the distribution of z corresponding to the degrees of freedom of the experiment. Thus the statement that z for a particular comparison > 1 per cent. point means that the value for z would be realised less than once in 100 times by chance. Similarly, if $z > 5$ per cent. point, that value would be realised less than once in twenty times by chance; the 5 per cent. point is usually accepted as implying a significant difference in the comparisons. It will be seen that the majority of our experiments satisfy the more stringent test.

Our experiments upon single bushes have given estimates of the healthiness of the bushes before and after treatment. The figures are therefore suitable material for an analysis by the method of covariance, which has been carried out for us by Dr R. A. Fisher personally. The influence of the start values upon the end values is assessed by estimating the regression, b . The corrected end value used is the actual end value less b times the start value. Upon these corrected values the final analysis of variance may be carried out. For tabulation however we have scaled these figures to a percentage of the difference between the values that would have been given by the average bush at the start (a) had it become entirely healthy, and (b) had it remained unchanged. Thus the figures in our tables represent the mean increase in healthiness in the bushes receiving the several treatments, expressed as a percentage of the possible increase in healthiness.

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EXPLANATION OF PLATES III—VI.

PLATE III.

Tea yellows disease. Paintings by Miss H. M. Edwards.

Fig. 1. Early stage. Leaves normal size and texture, but mottled with yellow.

Fig. 2. Intermediate stage. Leaves small, uprolled, pale yellow, scorched at tip and margins. Many leaves shed. Internodes shortened.

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Fig. 3. Late stage. A single minute leaf at tip, all the remaining leaves having been shed. Axillary buds growing out into short shoots with small yellow leaves. Internodes very much shortened towards tip.

PLATE IV.

Yellows disease in the field.

Fig. 4. Bush in early stage of yellows. Leaves chlorotic, but growth not appreciably reduced.

Fig. 5. Later stage. Leaves small, yellow, uprolled. Many leaves shed.

Fig. 6. Later stage. All leaves shed except small yellow ones at the tips of shoots.

Fig. 7. Last stage. All leaves shed except at the tips of a few shoots. Many shoots dying back.

Fig. 8. Healthy bush. One year previously this bush was in the condition of figure 5. At this time it received treatment with sodium sulphate, and as a consequence recovered completely.

Fig. 9. Typical severe yellows disease area in Nyasaland tea. The bushes in the valley are mostly healthy. Those on the slope have mostly died or are severely affected with yellows.

PLATE V.

Experiments in branch absorption of salts.

Fig. 10. Two branches on a single diseased bush. A side shoot of the upper branch has absorbed a solution of potassium chloride, without effect on the growth. The lower branch, which 20 days previously was in the same condition as the upper branch, absorbed potassium sulphate. As a consequence the original small leaves became green and a normal healthy flush of new leaves has appeared. (The vertical stick is a support for the test-tubes containing the salt solutions.)

Experiments in water culture of tea.

Fig. 11. Tea seedlings, three months old, grown in complete nutrient. Leaves dark green, normal size and texture.

Fig. 12. Parallel series, grown in nutrient lacking sulphur. The first plant shows typical yellow mottling of the young leaves. The young leaves of the remainder are small, yellow and uprolled, and many are scorched at the margins.

PLATE VI.

Tea plants grown in water culture, deprived of sulphur.

Fig. 13. Plant No. 1 of fig. 12, 4 months old. The terminal bud has grown out and produced three minute yellow leaves, two of which have already been shed. The leaf scars may be seen in the photograph. $\times \frac{3}{4}$.

Fig. 14. Plant No. 2 of fig. 12, 4 months old. The young leaves are small, yellow, uprolled and one is scorched from the tip. Two yellow leaves have been shed. $\times \frac{3}{4}$.

Fig. 15. Plant No. 4 of fig. 12, 4 months old. Eleven days previously this plant had shed all its small yellow leaves except one, of which the terminal half was scorched. On this day potassium sulphate was added to the culture fluid. In consequence the surviving half of the one leaf had become green and the terminal bud had started into healthy growth. $\times \frac{3}{4}$.

Fig. 16. The same plant, $4\frac{1}{2}$ months old. The half-scorched leaf has been shed, but the new growth is of normal colour and texture. $\times \frac{3}{4}$.

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Fig. 4.



Fig. 5.



Fig. 6.



Fig. 7.



Fig. 8.



Fig. 9.



Fig. 11.



Fig. 12.



Fig. 10.



Fig. 13.



Fig. 14.



Fig. 15.



Fig. 16.

THE DEVELOPMENT OF ASSIMILATORY TISSUE IN SOLANACEOUS HOSTS INFECTED WITH AUCUBA MOSAIC OF TOMATO

BY F. M. L. SHEFFIELD, Ph.D., F.L.S.

(*Department of Mycology, Rothamsted Experimental Station, Harpenden.*)

(With Plates VII-IX.)

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I. INTRODUCTION.

AUCUBA mosaic of tomato produces on the leaves of a number of Solanaceous hosts a vivid yellow mottling(13). The cells of the yellow areas are found to be practically devoid of chloroplasts, but it was not known how this condition arose. As this distinct mottling is confined to those leaves which were in active growth at the time of inoculation or which are produced later, it seemed unlikely that the plastids are destroyed, but rather that their development is in some way inhibited. The mode of origin of plastids in higher plants has long been a controversial point: it was therefore necessary to examine the normal course of development of the plastids before any attempt could be made to discover how the conditions existing in the diseased leaves are brought about.

Many virus diseases are characterised by the production in the cells of the host of abnormal inclusion bodies. In several Solanaceous hosts infected with aucuba mosaic of tomato, these bodies are built up by the gradual agglomeration of minute protein particles which appear in the

cytoplasm soon after infection(12). It was not known whether these bodies are present in the primary meristem. If they are present it would be interesting to know what is their behaviour at cell division.

Both the question of plastid development and that of the formation of intracellular inclusions involved an examination of the younger tissues of the shoot, and so work on the two problems was carried on more or less concurrently.

II. MATERIAL AND METHODS.

Three host plants, *Solanum nodiflorum*, *S. lycopersicum* and *Nicotiana tabacum*, were studied. Seedlings were infected with aucuba mosaic by leaf mutilation. The minute structure of these three hosts is identical and they are similar in their reaction to the virus disease; they will therefore be dealt with together.

Of the two problems mentioned above, work on the second, that of the virus inclusion bodies, was actually commenced before the former problem developed. Some of the methods employed to study intracellular inclusions were not applicable to the work on plastid development, and others had to be modified when this problem came under consideration.

(1) *Vital methods.*

In order to examine the young growing tissue of the shoot the material was sectioned, sometimes by hand but, more usually, by a freezing microtome. Sections were cut first at a thickness of 10μ for intracellular inclusion bodies and later, when a special study of the plastid development was being made, at 5μ . Sections were mounted in an isotonic sugar or salt solution. The results obtained by the study of living material were very disappointing. Owing to the rapidity with which death occurs in sectioned material, only a very cursory examination could be made. Also, in the living cells it is difficult to distinguish between plastid primordia and other granular cell inclusions and, as Randolph(10) and Zirkle(19) found, vital stains are of very little use. Recourse has therefore to be taken to more orthodox cytological technique.

(2) *Fixing and staining methods.*

Fixatives such as Zenker's and Flemming's fluids gave well-preserved nuclei and virus inclusion bodies in the younger plant tissues. Preparations from material fixed in this way were stained with Feulgen's fuchsin sulphurous acid stain and counter-stained with orange G, methyl blue or light green.

Although mature plastids are preserved, their primordia are destroyed by fixatives containing acetic acid; the latter are, however, preserved by bichromate mixtures. For the preservation of minute cytoplasmic inclusions, a number of methods were tried. These included Flemming's fluid without acetic acid, Altmann's fixative, osmic acid, Champy's solution and Champy followed by osmic impregnation. No perfect fixation over a whole preparation is ever obtained, for the degree of excellence varies greatly in tissues differing only slightly in development. The most generally reliable results were given by Champy's mixture, and this was used principally. The nuclei were not well preserved, but the proplastids and, when present, the virus inclusion bodies were quite well fixed.

The stem tips surrounded by a few of the youngest leaves were immersed in alcohol immediately on cutting from the plant to remove air held by the hairs and between the leaves. After 1 min. they were transferred to the fixing solution and the remaining air was removed with a suction pump. After all air was exhausted from the material the fixing solution was renewed, since volatile constituents such as osmic acid tend to be lost as the air is drawn off. The material was fixed for 24 hours and was then washed for a similar period in running water: it was dehydrated in alcohol, cleared in cedar-wood oil and embedded in 52° C. paraffin wax. Some sections were cut at a thickness of 10 μ for intracellular inclusion bodies, but thinner sections are of more value for the study of plastid development. Most of the material was cut therefore at 5 μ . A number of stains were used: Altmann's aniline fuchsin differentiated with picric acid and Haidenhain's haematoxylin gave the clearest preparations.

III. DEVELOPMENT OF CHLOROPLASTS IN NORMAL PLANTS.

The plastids can be traced back to a group of minute bodies present in the primary meristem of the shoot, which in plants of each of these three species consists of a number of small undifferentiated cells separated by very delicate walls with no intercellular spaces. Each cell contains a large nucleus embedded in the cytoplasm. The cytoplasm practically fills the cell, the few vacuoles which are present being quite small (Plate VII, fig. 1 and Plate IX, fig. 1). It is this lack of vacuolation and the fineness of the cell walls which render the young growing tissues so much more easily fixed than adult tissues.

Even at this early stage certain minute inclusions are present in the plasm and can be viewed in the living tissue. In each cell is a large number of minute slightly elongated granular bodies which are of the

order of 0.1μ in diameter (Plate VII, fig. 1 and Plate IX, fig. 1). They are of a highly reactive nature and consequently are easily destroyed in the processes of fixation and embedding. Normally they are preserved only by bichromate mixtures such as Champy's fluid. They stain black with Haidenhain's haematoxylin and red with Altmann's aniline fuchsin. It is to some of these bodies that the origin of the chloroplasts can be traced. It now seems fairly well established that plastids in the higher plants are formed either from chondriosomes or from bodies strongly resembling them. The literature has been frequently and ably summarised (5, 8, 11, 16, 17), and does not need to be discussed here. These minute cytoplasmic inclusions present in the cytoplasm of the cells of the primary meristem appear to be all identical and can be differentiated neither by their form nor by their chemical reactions. No evidence was forthcoming to indicate whether certain of these bodies are pre-ordained to develop into chloroplasts or whether their further development is a matter of pure chance.

An ergastic substance is often found in these tissues (Plate IX, fig. 2), but it is sharply differentiated from the primordia or chondriosomes by its chemical reactions. It takes the form of globules which vary considerably in size, the smallest being of the same order as the plastid primordia. The globules are particularly abundant in the epidermal cells, but are usually absent from the more deeply seated tissues. They are highly refractive and are preserved alike by acetic and bichromate mixtures which render them insoluble during the processes of dehydration, clearing and embedding. They stain black with Haidenhain's haematoxylin, yellow with Altmann's stain, green with aniline fuchsin methyl green, yellow with Feulgen and orange G, green with Feulgen and light green. They blacken with osmic acid, with Sharlach R they give a pink coloration and a faint pink reaction with Millon's reagent. They appear to be oil bodies of the nature of a food substance. In slightly older tissue they disappear.

Cell division in the meristem is frequent and rapid. Prior to each mitosis the cytoplasmic inclusions become segregated into two groups, one around each pole of the spindle, so that roughly half of the bodies are included in each daughter cell. It was not known whether the chondriosomes themselves divide or whether they arise *de novo* in the plasm. No direct evidence of division was obtained, although a paired arrangement, suggesting division, is often seen.

After active cell division ceases the cells, having become differentiated to form the various tissues of the leaf, increase in size. At the same time, the primordia of the chloroplasts also begin to increase in size in those

cells which are destined to become assimilatory tissue (Plate VII, figs. 2-3). Soon a small vacuole appears in the centre of each primordium or, if the primordium is a slightly elongated one, the vacuole may appear towards one end (Plate VII, fig. 4 and Plate IX, fig. 3). These bodies rapidly increase in size and now fix as small spheres with a large central vacuole and a dark staining periphery (Plate VII, fig. 5). About this time a slight pigmentation of the peripheral layer can sometimes be seen in living tissues. The vacuole contains a starch grain which cannot ordinarily be seen in fixed preparations, as the index of refraction of the starch is almost the same as that of the mounting medium. In the living cell, however, the grains give a dark coloration with iodine and show the characteristic starch structure if examined in polarised light. The proplastid continues to increase in size and minute pores appear in the dark staining stroma which surrounds the starch grain. Thus communication is established between the central vacuole and the exterior of the plastid (Plate VII, fig. 6). Throughout its development the plastid gradually becomes more and more resistant to chemical reagents, and some time before it reaches maturity it is capable of being preserved by acetic as well as by bichromate mixtures.

The development of the plastids of these Solanaceous plants conforms very closely to the mode of development of the plastids in a number of other higher plants including *Elodea canadensis* (18), *Zea mays* (10, 18, 19) and *Pisum* (9).

The plastids have now assumed the form of the type chloroplast of the higher plants described and figured by Zirkle (17), part II, Plate XXVII, fig. 45). Each is a hollow body, spherical or ellipsoidal in form. Within the central vacuole is a starch grain: two or even three grains may appear later (Plate VII, figs. 6, 7). The vacuole is enclosed by a pigmented stroma which takes a deep stain and which is penetrated by innumerable minute pores. The plastids are embedded in the cytoplasm, usually in the layer which lines the cell wall.

A mature plastid is of the order of 5μ in diameter. The size may vary with the amount of starch contained, and sometimes they swell so as to form an almost continuous layer at the periphery of the cell. If much swelling occurs the stroma forms merely a dark staining meshwork around the starch grain. Mature chloroplasts occasionally divide (Plate VII, fig. 8), but no direct evidence of division prior to this stage was obtained. A paired arrangement (Plate VII, fig. 2) of the plastid primordia was frequently observed and may result from division.

The development of the plastids is not intimately bound up with the

development of the cell, indeed, proplastids of many different stages may be found in the same cell. Their development usually commences as the cells become differentiated. In a young leaf the cells towards the lower surface, that is the spongy tissue, are often further advanced in development than those towards the upper surface which are destined to become palisade cells. Usually the plastids in the lower cells are more advanced than those in the upper undeveloped cells: this may be due to the relatively larger amount of light received by the ventral surface of the leaf at this stage. The plastids in the palisade cells may be fully developed before the cells elongate, but at other times quite young primordia are found in elongated cells. Plastid development is often further advanced towards the tip of the leaf which, again, is probably due to the larger amount of light received. Although, generally, the more advanced cells contain more fully developed plastids no rigid law is adhered to.

IV. CHLOROPLASTS OF DISEASED PLANTS.

When a plant becomes infected with aucuba mosaic a yellow mottling appears on those leaves which are in active growth at the time of inoculation and on leaves which are developed subsequently. These yellow areas are almost devoid of chlorophyll. Older leaves may show a certain amount of yellowing, possibly due to ageing of the plastids, but the distinct mottling never appears on leaves which are fully developed at the time of inoculation.

In section the differences between green and chlorotic areas are striking. A section through a green area, apart from intracellular inclusion bodies which may be present (p. 65), appears exactly like a section of a normal leaf. The green parts of the leaf contain several layers of palisade tissue, packed with chloroplasts, and below, several layers of spongy parenchyma also containing an abundance of green plastids (Plate IX, fig. 4). A section through a yellow area may show a few isolated cells filled with quite normal chloroplasts, but the majority of the cells are entirely devoid of plastids (Plate IX, fig. 5). The cells may be of the same size as those of the green areas, but more usually they are smaller. Often the palisade cells are not elongated at all and there are no intercellular spaces between parenchymatous cells. In these extreme cases, the chlorotic areas of the leaf are much thinner than are the green parts. The cells of the yellow areas often seem to contain more than the normal amount of cytoplasm.

Certain previous workers have stated that the plastids are destroyed by the virus, and an organism resembling a protozoon has been figured actually entering the plastids of plants with tomato mosaic (2). In

another paper the gradual dissolution of the plastids of diseased plants is described (15). The latter results would be more convincing if confirmed by the use of different technique, as the phenomena described might be brought about by keeping the sections in water for prolonged periods. Such treatment invariably causes the precipitation of hyaline spheres from the cell sap even in the abundance in which they are described in the hairs. Chloroplasts from normal plants if mounted in water soon swell up, each forming a large hyaline vesicle which may burst, or a number of them may form a state of equilibrium in the cell. As *aucuba mosaic* does not affect the chloroplasts of leaves which are fully developed at the time of infection, it seems unlikely that mature plastids are destroyed by the virus. The mottling of only young growing leaves suggests that the virus prevents the formation of plastids, and this has been found to be the case.

The meristematic tissue of diseased plants appears to be like that of healthy plants. Nuclear division is normal as is the structure of the cells. When cell division ceases the plastid primordia of certain of the cells begin to increase in size, and all stages in development of the plastids can be seen to occur exactly as in the normal plants (Plate VIII, figs. 1, 2; Plate IX, figs. 7, 9). But now other cells are seen to be devoid of proplastids. Sometimes a few minute dark-staining inclusions which may be either chondriosomes or primordia are present, but they do not develop further (Plate VIII, fig. 3 and Plate IX, fig. 9). It is evident that the virus has inhibited the development of the primordia and in many cases has completely destroyed them.

Various conditions are found within the adult cells of a diseased leaf. We will omit for the moment the question of intracellular inclusions caused by the virus. The cells of the green areas appear like those of the normal plants, but in the chlorotic areas cells may be of normal size and contain a few plastids and occasionally a few primordia or chondriosomes; they may be of normal size, containing no plastids but possibly a few undeveloped primordia; or they may be small when usually they contain primordia. The actual condition of the cells of a diseased leaf probably depends on the exact stage of development which has been attained when the virus reaches the cell in sufficient concentration to affect development. If the vacuolation of the primordia has commenced before the attack of the virus then it is very rarely that its further development is in any way affected. Occasionally proplastids of this stage are affected as is shown by the presence together in adult cells of bodies which may be chondriosomes or primordia, developing proplastids and very small mature plastids (Plate VIII, fig. 4 and Plate IX, fig. 8). If the virus reaches the

cell before plastid development has commenced, then the development of the plastids is inhibited and usually they are destroyed. Also further enlargement of the cell is prevented.

It has already been stated that the development of the plastids in the normal plants is not intimately bound up with the development of the cell (p. 61). For instance, plastid development may occur in a palisade cell before the cell commences to enlarge or it may not begin until the cell has elongated considerably. Thus in an infected plant sometimes palisade cells are found not to develop at all and in others the cells, although much elongated, are devoid of plastids. Further, a cell may contain primordia of several stages and, if this is attacked by virus, only the more advanced may complete their development.

The cells towards one side of a diseased leaf may be undeveloped, whilst those towards the other side are large and contain plastids. When this occurs it is almost invariably the upper cells which are affected. Presumably this is due to the fact that plastids develop earlier towards the lower side of the leaf. By the time the virus reaches the particular area of the leaf, the proplastids in the lower cells may be quite advanced, although those in the adjacent upper cells are still minute primordia which are easily destroyed by the virus.

Chondriosomes and plastid primordia are identical in all their reactions so far as can be observed. There is no evidence that they are not actually identical, it being a matter of chance which chondriosomes develop into plastids. It is not surprising that they react as readily as they do to the virus, since the chondriosomes of many plants and animals are known to react very readily to all kinds of cell injury (3). The reaction of the chondriosomes or primordia to the virus appears analogous to their reaction to chemical substances. It is just at the stage that they begin to become resistant to fixatives that they are usually unaffected by the attack of the virus. It has been suggested that the virus causes an increased acidity in the host tissues (1) and, in this connection, it is worthy of note that it is the fixatives of lower pH value which destroy the primordia most readily.

It should be emphasised that no evidence whatever was found of the destruction of mature plastids. Very occasionally the development of proplastids is inhibited during its course, but usually if a primordium is not destroyed or its development inhibited in a very early stage, then it will give rise to a perfectly normal chloroplast. Those primordia whose development is not inhibited behave in all ways as do those of the normal plant. In mature palisade cells the chloroplasts lie at the periphery. Occasionally they have a tendency, even in the normal plant, to become

swollen, when they tend to form a complete layer within the cell wall. In diseased plants this tendency has been found, occasionally, carried to an extreme, plastids becoming so swollen with starch that they actually fill the cell, their pigmented stroma forming a meshwork between the grains (Plate VIII, fig. 5). This was the only abnormal behaviour seen in the mature plastids of plants with aucuba mosaic, and it is not established that it may not occur also in healthy forms.

V. INTRACELLULAR INCLUSION BODIES.

In a number of Solanaceous hosts including *Solanum nodiflorum*, *S. lycopersicum* and *Nicotiana tabacum* infected with aucuba mosaic, the first symptom of the disease to be evident in the adult cells is the accelerated streaming of the cytoplasm. Minute protein particles appear in the plasma which carries them passively about the cell. These particles aggregate and fuse and, in the course of a few days, a large inclusion body is built up (12). Protein inclusion bodies have been described as associated with a number of virus diseases of both plants and animals. In tobacco with mosaic disease, they are present in the meristem where they are thought to divide prior to nuclear division (4). No inclusion bodies could be found in the meristematic tissue of plants infected with aucuba mosaic. Their formation does not commence until after cell division has ceased and, when present, they are invariably formed by the aggregation of small particles. Those minute cytoplasmic inclusions, the chondriosomes, are swept into the body as it is formed and can be seen in preparations suitably fixed and stained (Plate VIII, fig. 6 and Plate IX, fig. 6).

The bodies are not confined to the chlorotic areas of the leaf but are formed equally abundantly in green and yellow tissues. They are particularly abundant in the hairs of the leaves of all three hosts, being present in practically every cell. Localised areas of epidermal cells containing bodies, occur in both green and yellow tissues. Very occasionally a few adjacent palisade or spongy parenchyma cells below the affected epidermis may contain bodies. This apparent random distribution of the bodies over chlorotic and "healthy" areas was not understood (12). If the virus was present in sufficient concentration to cause the production of bodies it seemed strange that the plastids could withstand it and if plastid development was inhibited why did the virus not of necessity cause the production of bodies? The apparent anomaly is explained by the delay in the formation of bodies until after the plastids are developed. The young cell responds in several ways to the virus attack. Cell growth is inhibited, simultaneously the proplastids are destroyed or their

development prevented and, later, intracellular inclusions may be formed. If plastid formation is not inhibited at a very early stage, normal plastids will be formed and persist even if the virus reaches the cell at a later stage. But the virus may not reach a cell until after its growth is almost complete and the plastids have become resistant: in such a case inclusion bodies may be formed although the plastids are unaffected.

It is difficult to account also for the relative prevalence of these inclusion bodies in tegumentary tissues and their rarity in the parenchyma. It was thought possible that their formation might be to a certain extent controlled by the pH of the cell contents. An attempt was therefore made to determine the relative acidity or alkalinity of the different tissues, Small's technique (7, 14) being followed. The method is not sufficiently delicate to give absolute values for such fine structures, but it was thought that certain tissues might be found to be more or less acidic than others. The results obtained were disappointing: there was very little differentiation between the tissues, although the tegumentary tissues tended to have a very slightly lower pH value than the other tissues. Differences between different hosts were marked, but no differences were found between healthy and diseased plants.

VI. SUMMARY.

The development of the chloroplasts in *Solanum nodiflorum*, *S. lycopersicum* and *Nicotiana tabacum* is described and comparisons are made with plants infected with aucuba mosaic.

In the normal plants after cell division ceases in the meristematic tissue certain minute bodies, which are present in the cytoplasm of all young cells, commence to enlarge. A vacuole is formed in each and this gets bigger as the proplastid increases in size. A starch grain is formed in the vacuole. The outer stroma becomes pigmented and pores are formed in it. Increase in size continues, the mature plastid being about 5μ in diameter. A second or third starch grain may be formed in the vacuole. Chloroplasts sometimes divide.

In plants infected with aucuba mosaic certain of the leaf tissues are devoid of plastids and the cells may be undifferentiated. The absence of chlorophyll is brought about by the inhibition by the virus of the development of the plastid primordia. Usually the primordia are destroyed. If plastid development is not prevented in a very early stage, perfectly normal plastids are formed. Mature plastids are never affected by the virus but occasionally intermediate stages may be.

Intracellular inclusion bodies are not found in meristematic tissue, but incipient bodies appear when the cells are increasing in size and after plastid development is well advanced. For this reason inclusion bodies are formed indiscriminately in green and chlorotic areas, the virus presumably having reached the green tissues too late to inhibit plastid development.

An attempt was made to determine whether the prevalence of intracellular inclusion bodies in tegumentary tissues and their rarity in assimilatory tissues is due to differences in the pH of the tissues but the results obtained were rather indefinite.

This work was carried out under the auspices of the Empire Marketing Board.

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EXPLANATION OF PLATES VII-IX.

The figures of Plates VII and VIII were sketched at table level with the aid of a Zeiss camera lucida. A Leitz apochromatic 2 mm. objective (N.A. 1.4) was used in combination with Leitz periplanat oculars. For Plate VIII, fig. 5, a 10 \times ocular was used giving a magnification of 1680 \times and for fig. 6, a 6 \times ocular gave a magnification of 1000 \times . All other drawings were made using a 15 \times ocular, a magnification of 2350 \times being obtained. All drawings are reproduced without reduction.

Objects are shaded in the drawings according to the plane in which they lie: usually those in top focus are black whilst those in lower foci are successively paler.

For most of the photomicrographs of Plate IX, a Leitz 2 mm. apochromatic objective (N.A. 1.4) was used in combination with Leitz compensating oculars 6 \times or 10 \times . Magnifications were further controlled by adjustments of the bellows of the camera. For figs. 4 and 5, a Leitz 6 L objective was used.

After each description is given, in brackets:

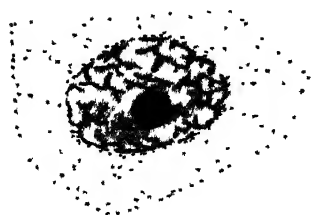
- (a) the fixative used for the preparation;
- (b) the stain used for the preparation;
- (c) the thickness of the section;
- (d) the magnification.

The following abbreviations are used:

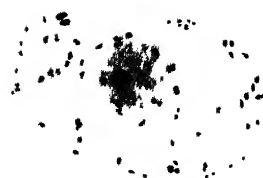
Ch. = Champy's fluid. Fl. = Flemming's fixative. F.P. = Aniline fuchsin destained with picric acid. H.H. = Haidenhain's haematoxylin.

PLATE VII.

- Fig. 1. *Solanum nodiflorum* healthy. Cell from meristem showing large nucleus and minute inclusions in the cytoplasm. (Fl. without acetic acid, H.H., 10 μ , 2350 \times .)
- Fig. 2. *S. nodiflorum* healthy. Cell from young leaf. Cell division has ceased and the minute cytoplasmic inclusions are enlarging. (Ch., H.H., 10 μ , 2350 \times .)
- Fig. 3. *S. lycopersicum* healthy. A similar stage to fig. 2. (Ch., H.H., 10 μ , 2350 \times .)
- Fig. 4. *S. nodiflorum* healthy. Vacuolation and enlargement of the proplastids in leaf cells. (Ch., H.H., 5 μ , 2350 \times .)
- Fig. 5. *S. nodiflorum* healthy. Cell containing proplastids in many stages of development. (Ch., H.H., 10 μ , 2350 \times .)
- Fig. 6. *S. lycopersicum* healthy. Young palisade cell containing small chloroplasts. Each consists of a hollow green sphere, containing starch in the vacuole. The stroma is porous. (Ch., H.H., 10 μ , 2350 \times .)
- Fig. 7. *S. lycopersicum* healthy. Mature chloroplasts showing minute pores in outer green stroma and starch grains contained in the central vacuole. The lowermost plastid was sketched in middle focus. (Living, unstained—2350 \times .)
- Fig. 8. *S. lycopersicum* healthy. Mature chloroplasts about to divide. The stroma has formed a meshwork owing to its porous nature and shrinkage in fixation. (Ch., H.H., 5 μ , 2350 \times .)



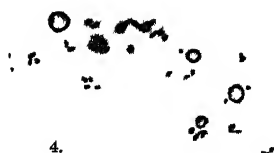
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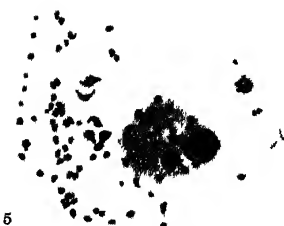
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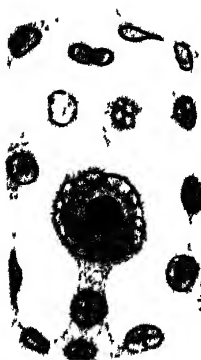
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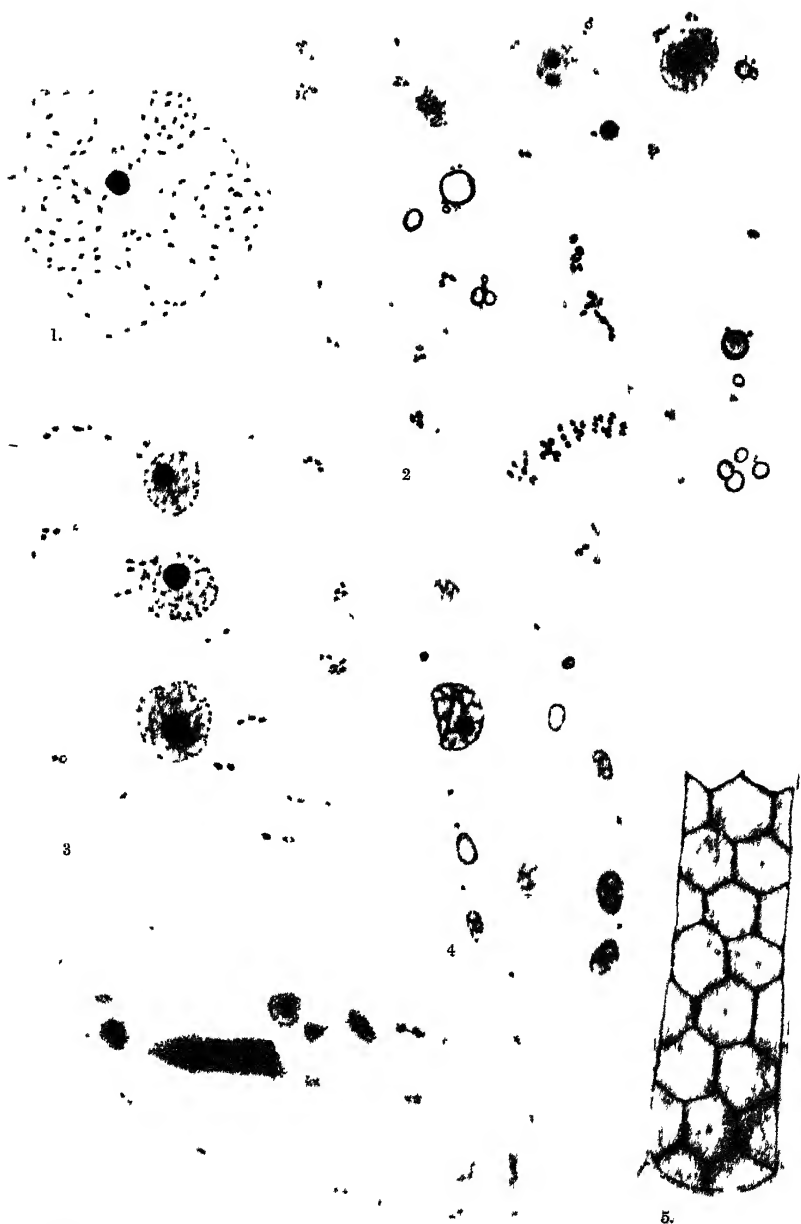


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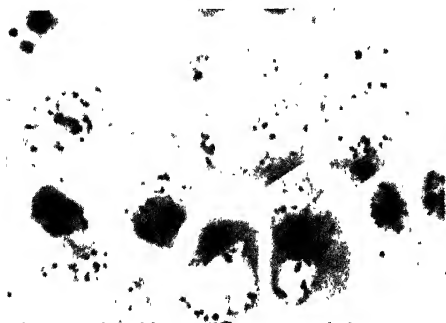
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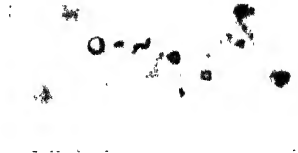
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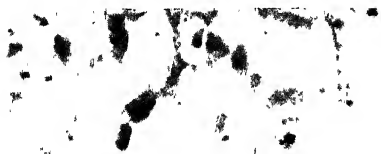
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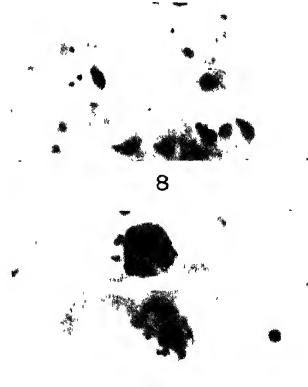
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8



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PLATE VIII.

- Fig. 1. *S. lycopersicum* with aucuba mosaic. Cell from young leaf. Karyokinesis has ceased, the cell and its minute cytoplasmic inclusions are enlarging. (Ch., F.P., 10μ , $2350\times$.)
- Fig. 2. *S. nodiflorum* with aucuba mosaic. Vacuolation and enlargement of proplastids in young cells of leaf. (Ch., F.P., 10μ , $2350\times$.)
- Fig. 3. *S. nodiflorum* with aucuba mosaic. Palisade cells from yellow area of leaf. There are no chloroplasts but the cells contain a few small cytoplasmic inclusions. (Ch., H.H., 5μ , $2350\times$.)
- Fig. 4. *S. nodiflorum* with aucuba mosaic. Cell from young leaf containing young plastids and also proplastids whose development has been inhibited. (Ch., H.H., 5μ , $2350\times$.)
- Fig. 5. *S. nodiflorum* with aucuba mosaic. Part of abnormal palisade cell. The chloroplasts are swollen with starch and fill the cell. (Ch., H.H., 5μ , $1680\times$.)
- Fig. 6. *S. nodiflorum* with aucuba mosaic. Intracellular inclusion body in process of formation in cell from hair of leaf. Several masses of material containing protein particles and chondriosomes are suspended in the cytoplasm. Later these masses will all fuse together. (Ch., F.P., 5μ , $1000\times$.)

PLATE IX.

- Fig. 1. *S. lycopersicum* healthy. Cells from young leaf showing minute inclusions in cytoplasm. (Ch., H.H., 5μ , $1460\times$.)
- Fig. 2. *S. nodiflorum* healthy. Axil of very young leaf showing oil bodies in epidermis of stem and leaf. (Fl., H.H., 5μ , $700\times$.)
- Fig. 3. *S. nodiflorum* healthy. Development of proplastids. Same field as Plate VII, fig. 4. (Ch., H.H., 5μ , $1460\times$.)
- Fig. 4. *Nicotiana tabacum* with aucuba mosaic. Transverse section of green area of young leaf. (Ch., H.H., 5μ , $300\times$.)
- Fig. 5. *N. tabacum* with aucuba mosaic. Transverse section of chlorotic area of same leaf as fig. 4. (Ch., H.H., 5μ , $300\times$.)
- Fig. 6. *S. nodiflorum* with aucuba mosaic. Intracellular inclusion body in hair cell. The body contains chondriosomes. (Ch., H.H., 10μ , $700\times$.)
- Fig. 7. *S. nodiflorum* with aucuba mosaic. Developing proplastids in young leaf. Plate VIII, fig. 2 shows part of this field. (Ch., F.P., 10μ , $1460\times$.)
- Fig. 8. *S. lycopersicum* with aucuba mosaic. Undeveloped plastid primordia, proplastids whose development has been suspended and small plastids all present in same cell. (Fl. without acetic acid, H.H., 5μ , $1460\times$.)
- Fig. 9. *S. nodiflorum* with aucuba mosaic. Palisade cells, devoid of plastids, from chlorotic area of leaf. (Ch., H.H., 5μ , $1460\times$.)

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ON THE USE OF THE PRIMARY LESIONS IN QUANTITATIVE WORK WITH TWO PLANT VIRUSES

BY GEOFFREY SAMUEL, M.Sc.

(*Waite Agricultural Research Institute, University of Adelaide.*)

AND J. G. BALD, B.AGR.SC.

(*Division of Plant Industry, Council for Scientific and
Industrial Research, Australia.*)

(With Plate X and 6 Text-figures.)

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INTRODUCTION.

It is now nearly four years since Holmes (1929 *a*) demonstrated that by the use of the necrotic local lesions produced by tobacco mosaic on certain species of *Nicotiana*, much more accurate quantitative work was possible than by the older method of systemic infection from needle inoculation. In the intervening time, however, no papers have appeared in which Holmes's method has been applied, and considering how great is the need for more reliable quantitative methods in virus work this is somewhat surprising. The reason may be partly that Holmes, although clearly indicating the value of his method, did not present many of the details which would be involved in any practical application of it. Or it

may be that virus workers in general were sceptical as to whether the method would be applicable in the case of other viruses, in almost none of which, up to that time, was the existence of definite primary lesions recognised.

It is the purpose of this paper to present a number of the details of which account must be taken in practical application of Holmes's method. The value of the method, moreover, will become more evident from a number of the experiments, which provide answers with definite statistical significance to some questions which it would have been impossible to investigate by the older methods. The virus of tomato spotted wilt has been used to check a number of the experiments, since it has been found to form definite primary lesions on the leaves of tobacco (as well as on several other species of *Nicotiana*). It seems probable that a number of other mechanically inoculable plant viruses will later be found to form definite primary lesions of a character suitable for quantitative work.

METHODS.

The two viruses used, tobacco mosaic 1, obtained from Prof. James Johnson, and tomato spotted wilt, offer a very great contrast in properties. Tobacco mosaic retains its infectivity for years in dried material or in expressed sap; it is highly infectious and can be diluted to one in a million and still cause infection; it has a thermal death-point of 88° C. for 10 min. heating; and it has considerable resistance to chemicals. Tomato spotted wilt, on the other hand, retains its infectivity in expressed sap for about 3 hours only at room temperatures; needle inoculation rarely, if ever, succeeds; it cannot be diluted much above one in a thousand and remain infectious; it has a thermal death-point of only 42° C. for 10 min. heating; and it has a very low resistance to chemicals.

The rapid loss of infectivity of tomato spotted wilt virus in expressed sap made it an extremely difficult virus to work with. The change in concentration at room temperature, or even in freezing mixture, was rapid enough to complicate very considerably the comparison of different lots (from dilutions, thermal treatments, etc.), owing to the fact that they could not all be inoculated simultaneously.

The provision of a continuous supply of the virus of tomato spotted wilt of sufficient potency for experimental work was also a matter of difficulty, since the concentration of virus in diseased plants falls so rapidly (see p. 92). This was overcome by inoculating batches of plants every few days, so that there was available a constant supply of plants

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which had become diseased in the week preceding any experiment. In summer and autumn a considerable amount of virus was available from freshly diseased field plants.

The methods used followed those of Holmes, with certain modifications introduced as the work proceeded. For measurements of tobacco mosaic, plants of *Nicotiana glutinosa* trimmed to five leaves were used. For measurements of tomato spotted wilt, tobacco plants (variety Blue Pryor mainly, but White Burley is as good) were inoculated on two or three leaves each, when the plants were just at the end of the rosette stage and commencing to form the stem. At first younger plants in 4-in. pots were used, but owing to the rapid drying out in summer 6-in. pots were used later, and the inoculated leaves were often 8 in. or more in length. Precautions which should be observed in raising batches of uniform plants are discussed in the section on *Condition of plants*. Little quantitative work could be done with tobacco mosaic in the summer months on account of the rapid extension and coalescence of the primary lesions on *N. glutinosa* at high temperatures.

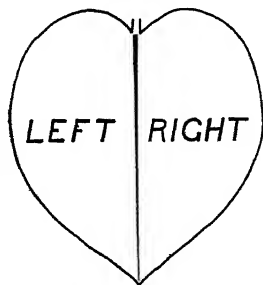
At first inoculation was done by wiping the surface of the leaf with muslin dipped in virus, but for several reasons this method was soon abandoned in favour of the ground glass spatula (Samuel, 1931). The spatulas were improved and made longer in the blade to fit across half a leaf of *N. glutinosa*. The details of inoculation are further described on p. 91. In the case of tomato spotted wilt, when inoculations had to be done very rapidly to avoid appreciable ageing, the muslin wiping method was sometimes used instead of the spatula because it was somewhat faster.

Counts of primary lesions were usually made 6-8 days after inoculation. Holmes speaks of local lesions of tobacco mosaic on *N. glutinosa* appearing on the second or third day after inoculation, and final counts being made on the fourth or fifth day. In our experiments the primary lesions did not show up so quickly, probably because of the lower temperatures in our unheated greenhouse. The lesions usually appeared on the third to the fifth day, and progressive daily counts on plants kept at medium temperatures showed that the numbers did not increase appreciably after the eighth day. It is a matter of temperature, however, and in winter time the plants had to be left longer before counting than in spring or autumn. The primary lesions of tomato spotted wilt on tobacco have similar time relations.

In counting it was found convenient to count and record the two sides of the leaves separately. Where considerable numbers of lesions were

present a system of following down between lateral veins had to be adopted to avoid error. The sides of the leaves were named as in Text-fig. 1.

In the statistical work the standard error has been used throughout instead of the probable error. The test for significance is therefore that a difference should exceed twice the standard error¹. In comparisons by the half-leaf method each plant was considered an experiment, and the figures for the left and right half-leaves totalled separately. The differences in a number of replications (usually five or ten plants) were then treated as a set of paired experiments, and the odds for significance worked out from Fisher's (1928) table of *t*, or (when given in the paper as definite odds) from the tables given by Love (1924) in his modification of Student's method.



Text-fig. 1.

THE DILUTION CURVE.

Tobacco mosaic.

Since it was desired to make the comparison of virus concentrations as simple as possible consistent with statistical significance, the first experiment was a repetition of Holmes's dilution test in order to examine the variation to be expected, the number of plants which should be used, etc. Batches of ten trimmed *Nicotiana glutinosa* plants were inoculated by the muslin-wiping method with each dilution of a freshly extracted tobacco mosaic virus, and the counts of the primary lesions are summarised in Table I.

Table I.

Showing the number of primary lesions produced on each of ten N. glutinosa plants inoculated with the dilutions of tobacco mosaic shown.

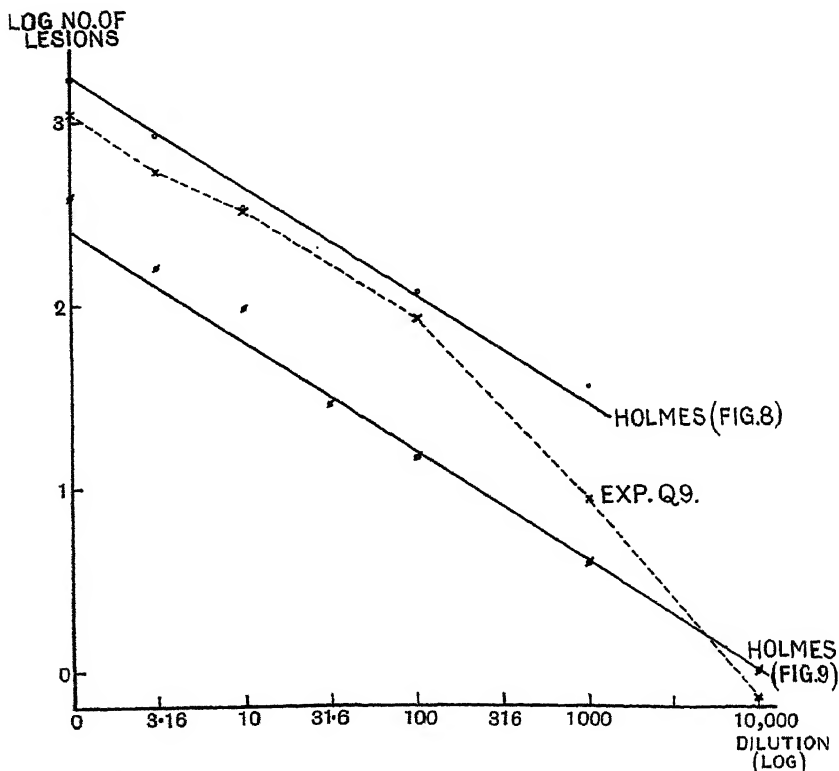
Undiluted	1:√10	1:10	1:100	1:1000	1:10,000
1322	283	235	80	5	0
1154	239	414	54	7	1
1232	802	202	103	12	1
910	545	214	84	2	4
908	848	202	126	10	1
1182	397	209	70	15	0
1241	641	566	143	12	0
760	482	396	37	13	0
1040	406	396	60	12	0
1292	693	487	103	0	0
Means	1104±60	534±66	332±43	86±10	8.8±1.6
					0.7±0.4

¹ The formula used for calculating the standard error of the means was:

$$\text{Standard error of the mean} = \sqrt{\frac{\sum (x - \bar{x})^2}{n(n-1)}}$$

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The results are plotted to a logarithmic scale in the dotted curve of Text-fig. 2, and in the same figure two of Holmes's (1929 *a*) experiments are plotted, using the completely logarithmic scale instead of the semi-logarithmic scale used by Holmes. Unfortunately, Holmes did not give the actual figures obtained in his experiments, so that the points plotted had to be read off as accurately as possible from his graph.



Text-fig. 2. Dilution curves for tobacco mosaic, plotted on a logarithmic scale. Data from two of Holmes's experiments transposed to this scale, and the line of slope 0.6 superimposed in both cases.

Superimposed upon Holmes's points has been placed in each case the straight line with slope 0.6, which corresponds to a halving of the number of primary lesions with each successive dilution: $10^{\frac{1}{2}}$, 10, $10^{\frac{3}{2}}$, 10^2 , $10^{\frac{5}{2}}$, It is noticeable at once how marked is the agreement between the points and this line, although in the lower curve the three greatest concentrations have given values considerably too high. In

our own experiments also there is a decided tendency for the points to lie along a line of the same slope in the upper portion, but serious discrepancies occur in the two lower points.

It seemed advisable, therefore, to examine carefully the experimental error to be expected in plotting points by this method. Table II gives full details of the counts for the individual plants in the case of one of the dilutions ($1 : 3.16 = 1 : \sqrt{10}$).

Table II.

Showing the numbers of primary lesions produced on each half-leaf, leaf, and whole plant in a series of ten N. glutinosa plants inoculated with the virus of tobacco mosaic diluted $1 : 3.16 (= 1 : \sqrt{10})$.

Plant No.	No. of lesions on half-leaves*					No. of lesions on whole leaves					Total per plant
	(1)	(2)	(3)	(4)	(5)	(1)	(2)	(3)	(4)	(5)	
1	57	31	23	33	34	83	51	42	58	49	283
2	26	20	19	25	15						
3	15	24	23	24	23	33	43	55	48	60	239
4	18	19	32	24	37						
5	52	35	104	145	124	78	67	208	245	204	802
6	26	32	104	100	80						
7	30	43	98	81	47	58	75	196	127	89	545
8	28	32	98	46	42						
9	38	77	84	215	84	68	136	174	319	151	848
10	30	59	90	104	67						
1	35	40	46	32	60	80	85	79	73	80	397
2	45	45	33	41	20						
3	38	24	38	121	111	65	47	90	196	243	641
4	27	23	52	75	132						
5	36	27	49	66	77	83	69	78	127	125	482
6	47	42	29	61	48						
7	32	41	53	56	51	78	71	87	89	81	406
8	46	30	34	33	30						
9	46	36	101	125	72	74	72	169	205	173	693
10	28	36	68	80	101						
Mean										533.6 ± 66.2	

* Count for left half-leaf above, right half-leaf below, in each case. Leaves numbered (1) to (5) from the bottom of the plant to the top.

The total number of lesions per plant varies from 239 up to 848. This large variation appears to be due to a difference in susceptibility of individual plants rather than to random error, for in the case of plants with high totals, such as No. 5, the count on every leaf is consistently high, and similarly plants with low totals, such as No. 2, have low counts for every leaf. Even with ten replications the standard error of the mean (534 ± 66) is still 12.4 per cent. The next dilution ($1 : 10$) had a mean 332 ± 43 . The difference between the means is therefore 202 ± 79 , which is significant, but not very greatly so. It is obvious, therefore, that the position of the points on the graph may vary rather widely due to experimental errors. This raised the question as to whether improvements in

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technique could not be introduced which would considerably reduce this error.

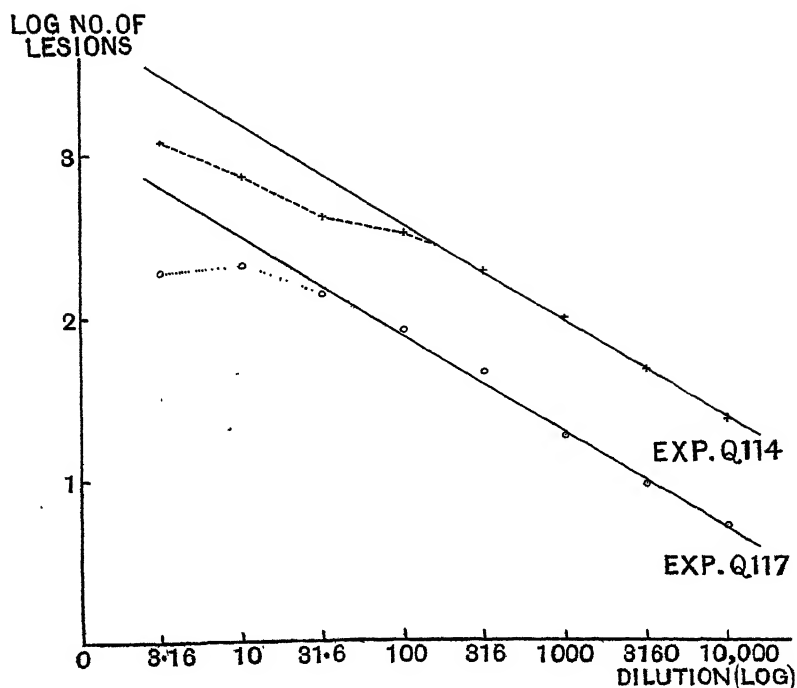
For ease of counting, each half of each leaf was counted separately (column 2, Table II). It is seen that there is a much greater correspondence between the counts on each side of the same leaf than there is between different leaves or different plants. It is in comparatively few cases that one half of the leaf has more than twice as many lesions as the other. In thirty-three cases out of the fifty, however, the number of lesions on the left-hand side of the leaf exceeds that on the right; in four cases they are equal, and in thirteen cases less. When the differences are examined by Student's method it is found that there is a mean difference in favour of the left-hand side of 11.5 ± 3.3 lesions, which is quite significant. This may have resulted from a greater pressure of the end joint of the forefinger over the left-hand side of the leaf during inoculation, but whatever its causes it is a point to which attention should be given in devising a more accurate technique. The further examination of these points is dealt with in subsequent sections. Here it may be stated that a more accurate technique of inoculation has been developed, the viruses which are being compared being inoculated with a ground glass spatula on opposite sides of the same leaves.

Using this method the dilution tests have been repeated, each dilution being compared on opposite halves of every leaf of ten plants with the immediately preceding and the immediately following dilution, according to the plan:

1 : 3.16	1 : 10	1 : 31.6	1 : 100	1 : 316	1 : 1000	1 : 3160
v.	v.	v.	v.	v.	v.	v.
1 : 10	1 : 31.6	1 : 100	1 : 316	1 : 1000	1 : 3160	1 : 10,000

Two of the curves obtained in experiments of this type are presented in Text-fig. 3. It is again noticeable how closely the points fit a curve of slope 0.6 in the lower ranges of dilution. With the stronger concentrations of virus, however, there is a marked falling away from this line. In Table III are shown the actual figures obtained in Exp. Q 114. Since the totals for each dilution are the numbers of lesions formed on half-leaves of twenty plants they are equivalent to the number which would have been formed on the whole leaves of ten plants. Dividing by ten therefore, gives the average number of lesions which would have been formed per plant by each dilution.

In column C, Table III, is given a hypothetical series which would conform to a line of slope 0.6 along its whole length, and it is seen that if



Text-fig. 3. Dilution curves for tobacco mosaic from experiments planned on the half-leaf method. The line of slope 0.6 superimposed in each case.

Table III.

Showing the number of lesions produced on N. glutinosa plants inoculated by the half-leaf method with the dilutions of tobacco mosaic shown (Exp. Q. 114).

Dilution	Nos. of lesions on		C
	10 half-leaves	20 half-leaves	
Undiluted	Omitted		6400
1: 3.16	6377 5787	12,144	3200
1: 10	4655 2815	7,470	1600
1: 31.6	1978 2336	4,314	800
1: 100	1793 1570	3,363	400
1: 316	951 1059	2,010	200
1: 1000	513 490	1,003	100
1: 3,160	196 281	477	50
1: 10,000	80 154	234	25

there were twenty-five lesions per plant at dilution 1 : 10,000 there would need to be 6400 lesions per plant, or over 1200 per leaf, with undiluted virus. Crowding of lesions to this extent has never been seen to occur, and it seems probable that at the higher dilutions some other factor, such as number of suitable entry points for the virus, comes in as a limiting factor. It is rather surprising that there is no evidence of this falling off in the number of lesions at the higher concentrations in the curves presented in Fig. 2. It appears possible, however, that Holmes was working with a dilute virus in the case presented in his Fig. 9, for his scale of dilutions does not conform to those given in his Fig. 8. His other curve is also difficult to interpret, for he plots 1714 lesions per plant for the undiluted virus, but remarks on the opposite page that "undiluted tobacco mosaic extracts result in the production of about 300-600 lesions on each test plant." Our own virus, in our first experiment, gave less than one local lesion per plant at 1 : 10,000 dilution, so that it was a considerably weaker virus than that used in Exp. Q 114, in which over twenty-three local lesions per plant were formed at this dilution. At all events, all the dilution experiments which we have done using the more accurate half-leaf method, with tobacco mosaic on *N. glutinosa*, have given the type of curve shown in Text-fig. 3.

It is a point of considerable interest that the slope of the curve in the lower ranges of dilution has tended to the value 0.6 in all the experiments, both those of Holmes and our own. This would mean that the number of lesions in this range varied as the fifth root of the dilution cubed. It would be unwise to assume that there was any such relation between the number of free virus particles and the dilution, however. This is the value obtained when tobacco mosaic is inoculated on *N. glutinosa*, but it might not be the value obtained using some other species of *Nicotiana*. In fact, if the graph given by Holmes in Fig. 3 of his paper on "Accuracy in quantitative work with tobacco mosaic virus" (1928) (which was compiled from dilution studies using the insect pin inoculation method on tobacco seedlings) is drawn to a completely logarithmic scale using the number of successful infections instead of the mean reduction, then the curve showing the relation between infections and dilution has a slope of 0.15. It is evident that further study of the dilution curve, as drawn by different methods, may prove of considerable interest.

Tomato spotted wilt.

The virus of tomato spotted wilt when expressed from the plant loses its infectivity so rapidly that it was found impossible to carry through a dilution experiment on the plan used for tobacco mosaic. Since it was very desirable to obtain at least an approximate curve two methods were considered of overcoming the difficulty. One was to make the desired dilutions as rapidly as possible and to have a number of operators inoculate them simultaneously, and the other was to determine whether from a number of diseased plants samples of leaves of approximately the same virus content could be chosen by eye, and to use from such a uniform batch of material, fresh samples for each successive dilution.

Three experiments have been done, the details of which will not be reported at this stage since it is hoped to introduce further improvements in method. The results of these experiments, when plotted on a logarithmic scale, agreed in indicating a straight line relationship between number of lesions and dilution in the lower ranges. The slope of the line in each case was about $\frac{2}{3}$. There was also some indication of a rise in the number of lesions with moderate dilution.

THE HALF-LEAF METHOD.

It was pointed out in the previous section that owing to the great variation between individual plants comparisons between the two sides of the same leaf yield much more significant results than do comparisons between different plants. The principle is the same, of course, as that which led Sachs to adopt the half-leaf method for determinations of carbon assimilation. In the case of experimental work with viruses, however, there is an added disturbing factor—the technique of inoculation. It was evident from the example given previously that with the muslin-wiping method of inoculation there was a definite tendency for a greater number of lesions to appear on the left-hand side of the leaf. It is unlikely that this constant difference is due to any inherent difference in the two sides of the leaf, and the most probable explanation is that it is due to a different pressure exerted on the two sides during inoculation, owing to the unevenness of the forefinger. When the glass spatula method of inoculation is used this difference is greatly minimised, but not entirely eliminated. Table IV shows the results of inoculating ten plants on both sides of the leaves with a 1 : 100 filtered tobacco mosaic virus with the standard glass spatula technique described on p. 91. Here again thirty leaves out of the fifty show a greater number of lesions on the left-hand side of the leaf than on the right, but in this case the difference

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in favour of the left side is only 2.6 ± 2.4 , a result which is not statistically significant. Since the same tendency is manifest in other experiments in which both sides of the leaf are inoculated with the same virus it is probable that consideration of a larger number of cases would reduce the standard error and make the difference significant, but it is always small. The explanation may again be one of varying pressures, or possibly of varying angles at which the hairs are broken. It would not repay more detailed investigation at the present stage, however, for it can be so easily neutralised by alternating the inoculations, so that in the comparison of any two viruses *A* and *B* half the plants are inoculated on the left of the leaf with virus *A* and on the right with virus *B*, and the remaining half on the left with virus *B* and on the right with virus *A*. This method has been adopted throughout our work. Its effect in neutralising differences is well shown when the plants in Table IV are split into two groups and the lesions on the left sides of the leaves of the first five plants are added to those on the right sides of the other five plants and *vice versa*. The totals, 1736 and 1738, are in this case extraordinarily close, in spite of considerable differences in susceptibility between individual plants. The totals from another similar experiment were 2799 and 2810.

Table IV.

Showing the numbers of primary lesions produced on each half-leaf in a series of ten N. glutinosa plants inoculated uniformly by the glass spatula method on both sides of the leaves, with 1 : 100 filtered tobacco mosaic virus.

Plant No.	No. of lesions per half-leaf*					Per plant totals for		Totals for five plants	
	(1)	(2)	(3)	(4)	(5)	Left halves	Right halves	Left halves	Right halves
1	70	25	62	61	24	242	202		
2	46	32	86	28	10	64	109		
	10	34	8	6	6				
	15	71	4	11	8				
3	15	35	27	31	12	120	127		
	36	28	37	16	10				
4	8	38	35	18	11	110	90		
	9	14	25	27	15				
5	14	44	62	150	29	299	243	835	771
	9	42	50	120	22				
6	20	69	62	64	28	243	292		
	32	50	76	112	22				
7	49	70	52	11	5	187	131		
	59	19	42	9	2				
8	73	64	93	53	10	293	240		
	58	55	98	20	9				
9	16	37	10	6	3	72	84		
	13	51	13	4	3				
10	45	50	58	12	7	172	154	967	901
	40	48	42	18	6				
Totals						1802	1672	1802	1672

* Count for left half-leaf above, right half-leaf below, in each case. Leaves numbered (1) to (5) from the bottom of the plant to the top.

It is desired to present in this section, however, a direct comparison between the half-leaf method and the method with groups of plants as used by Holmes¹. A number of such experiments have been done, but the following example is one which involves a fairly exacting comparison, viz. between a 1 : 1000 and a 1 : 2000 dilution of a virus which was not particularly strong at the commencement.

Ten plants were inoculated all over with the 1 : 1000 dilution; ten other plants were similarly inoculated with the 1 : 2000 dilution; and ten others with the 1 : 1000 and 1 : 2000 on opposite halves of each leaf, the first five plants being inoculated with the 1 : 1000 virus on the left and the other five with the 1 : 2000 on the left. The results are summarised in Table V.

Table V.

Comparison of virus concentrations by the group method and by the half-leaf method, using tobacco mosaic on N. glutinosa.

No. of lesions per plant from inoculation with virus diluted		No. of lesions on left half-leaves versus no. on right half-leaves	
1 : 1000	1 : 2000	1 : 1000 v. 1 : 2000	
154	67	17	40
43	32	27	35
61	29	23	46
55	31	12	16
48	65	59	61
		1 : 2000 v. 1 : 1000	
91	58	48	33
98	16	42	25
156	23	52	34
63	47	40	27
50	64	46	28
Means	81.9 ± 13.4	Mean diff. in favour of 1 : 1000 virus	14.1 ± 2.3
	43.2 ± 6.1		

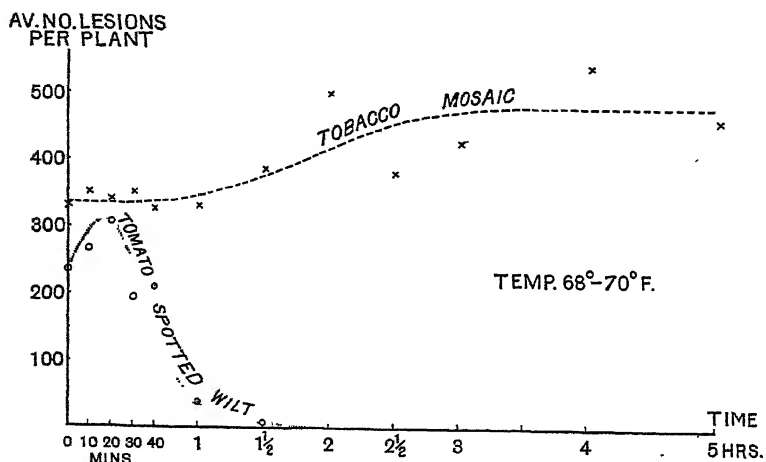
In the case of the groups of ten plants the difference between the means was 38.7 ± 14.7 . This corresponds to odds for significance of a little over 100 : 1, whereas the half-leaf method has given an answer with odds for significance of over 10,000 : 1, with only half the number of plants. In comparing two viruses, therefore, it is of much greater accuracy and a considerable saving in plants, to compare them on a number of opposite half-leaves, rather than on numbers of single plants. When the differences in concentration are at all marked each leaf can be considered a single experiment, and in many cases the ten leaves on two plants only will give an answer with statistical significance as to difference in strength of the two samples.

¹ Holmes (1929 b) also used the half-leaf method in an experiment on the effect of washing off the inoculum.

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When the method is used in larger experiments the manner in which it is applied must depend to some extent on convenience. For example, in the dilution experiments on p. 76 each dilution of virus was compared on half-leaves with the immediately stronger and immediately weaker virus. This gave a good degree of significance between successive points on the curve with the minimum of counting labour. A more accurate way would have been to compare each dilution with the strongest, but the counting involved would have been excessive.

The greater accuracy of the half-leaf method is also brought out graphically by a comparison of the curves for ageing of spotted wilt virus as drawn by the two methods (Text-figs. 4 and 5).



Text-fig. 4. Ageing curve for tobacco mosaic and tomato spotted wilt, from an experiment planned on the group of ten plants method.

APPLICATIONS.

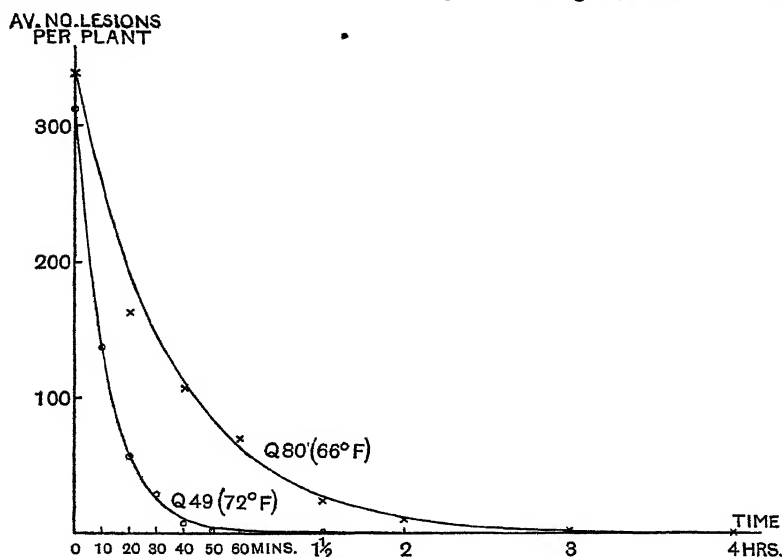
(1) *The standardisation of inoculating methods.*

Area of leaves.

One of the first points which arises in quantitative experiments is whether the area of the leaves of each group of plants should not be measured and comparisons be made on the basis of number of lesions per definite area of leaf surface, rather than on mean number of lesions per plant when plants are so variable. The half-leaf method practically eliminates the need for consideration of this factor in comparisons of only two viruses. Where more than two viruses are being compared, however, or where the group method is being used, the question of leaf

area must be considered. The calculation of the leaf area in every experiment would be quite an appreciable amount of work, and it would be an advantage if it could be avoided. It has been found that groups of plants for an experiment can be picked out by eye so that the standard deviation for area of the leaves is less than 2 per cent. of the mean.

There is always some variation in size in a large batch of plants raised for experimental work, and our procedure has been, if, say, eight even groups of plants are needed, to choose the eight largest plants first and place one in each group, then the eight next largest, and so on until



Text-fig. 5. Ageing curves for tomato spotted wilt from experiments planned on the half-leaf method.

the groups are complete. In an experiment in which four groups of ten plants were so chosen, and the leaf areas subsequently calculated by tracing and weighing with brown paper, the groups were found to have leaf areas of 513, 525, 513 and 526 sq. cm. respectively. The mean is here 519 ± 3.6 , and the standard deviation is 1.01 per cent. of the mean. Compared with other errors involved this is small, so that for most purposes the question of leaf area may be neglected, if the groups of plants have been properly chosen. This has been verified by calculation of the leaf area in larger experiments of the type presented in Text-fig. 5, and no appreciable alteration of the position of the points has resulted.

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For experiments in which different types of plants are being compared, such as nitrogen-deficient plants with small leaves as compared with healthy plants (see p. 87), calculation of leaf areas is, of course, essential.

Contamination from the under sides.

It has been found, both with tobacco mosaic on *N. glutinosa* and with tomato spotted wilt on tobacco, that just as many primary lesions may result from inoculating the under sides of the leaves as from inoculating the top sides. Experiments in which the top sides of the leaves were wiped with distilled water while the leaf was supported with fingers wet with virus showed that appreciable numbers of lesions could result from under side contamination. It is therefore important, in standardising a technique of inoculation, that contaminations from the under side be avoided. For this reason a piece of clean paper is held under each leaf during inoculation. Small squares of newspaper have been found quite sufficient for the purpose.

Glass spatula v. muslin.

The glass spatula method of inoculation was tested against the method of wiping with the finger wrapped in muslin in two experiments, using tobacco mosaic on *N. glutinosa*. The two methods of inoculation were used on opposite halves of the same leaves, with the usual precaution of alternating the sides. The results may be summarised as follows:

Exp.	Strength of virus	No. of plants	Mean no. of lesions per plant		Mean diff.	S.D. as % of mean	
			Glass spatula	Muslin		Spatula	Muslin
1	1:1000	20	105.8 ± 11.5	57.4 ± 7.5	48.4 ± 10.1	54.9	58.1
2	1:500	10	132.6 ± 17.8	85.0 ± 9.5	47.6 ± 10.6	42.3	35.4

It is seen that the glass spatula method gave a significantly higher number of lesions in both experiments. When the variation for each method of inoculation was examined by calculating the standard deviation as a percentage of the mean it was found that in one case the spatula method had a lower variation than the muslin, but that the reverse was true in the other case. The numbers, moreover, were so close in both experiments that it was evident that in the matter of variation there was no marked superiority of one method over the other.

Degree of rubbing.

An experiment was carried out, both with muslin and with glass spatula inoculation, to determine the effect of increasing the number of rubs over the leaf during inoculation. One rub, two rubs, four rubs and

ten rubs were all compared by the half-leaf method with two rubs. The experiment is too lengthy to report in detail, but if the figures are reduced to equivalence by equating the two-rub sides the following summary shows the trend of the results:

	Spatula	Muslin
1 rub	933	787
2 rubs	1350	1072
4 rubs	1761	1028
10 rubs	2260	1326

It was thought that if inoculation occurred mainly through broken hairs on the leaf the figures for four rubs and ten rubs might not be appreciably greater than for two rubs. It was found, however, that there was a significant, but by no means proportional increase in the number of lesions with increasing number of rubs, the effect being more pronounced with the ground-glass spatula inoculation than with muslin. It seems probable that inoculation through epidermal scratches may be entering in as a factor, but this point was not further investigated.

The variation was also estimated for each pair of inoculations to see whether one method gave a significantly smaller variation than the others. The figures for each pair were however of much the same order of magnitude. It was concluded that there was no definite number of rubs for inoculation, which showed an appreciable superiority over the others, and that as long as a definite standard was adopted the results would be comparable. Two rubs was the standard adopted in most of the work, with the idea that the variation might be reduced owing to the covering of small areas which might have been missed in the first rub, but as stated above, this was not found to be the case when it was tested. One rub should therefore be just as good a standard to adopt, and in the later part of the work was used because of the saving in time.

Washing off virus.

Holmes (1929 *b*) has already presented one experiment dealing with the effect of washing off the inoculum. In our work ten plants of *N. glutinosa* were inoculated uniformly with undiluted tobacco mosaic virus, and immediately after inoculating each plant the virus was washed off one-half of each leaf with a jet of water from a wash-bottle. The left half of each leaf was cleaned in the case of the first five plants, and the right half in the case of the remaining five. Another experiment was done at the same time, using virus diluted 1 : 100. The results may be summarised as follows:

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A. *Undiluted virus.*

Mean no. of lesions per plant, unwashed sides	484.7
" " " washed sides	547.9
Mean difference in favour of washed sides	63.2 ± 18.9
Odds for significance (Love's table)	216 : 1

B. *Diluted virus (1 : 100).*

Mean no. of lesions per plant, unwashed sides	81.8
" " " washed sides	96.0
Mean difference in favour of washed sides	14.2 ± 5.9
Odds for significance (Love's table)	49 : 1

In two other experiments similar results were obtained, except that no significant increase resulted from washing off the diluted virus. The above experiments support Holmes's suggestion that the increase in the number of lesions produced when undiluted virus is washed off is due to the fact that toxic effects of the plant juice on the slightly injured cells are avoided. The effect is much more marked when the virus is suspended in an injurious chemical, as illustrated by Holmes for virus in 50 per cent. glycerine. Where time is a consideration we have adopted the plan of immediately washing off any virus more concentrated than 1 : 10, or any virus suspended in a chemical, leaving plants inoculated with dilute viruses until the whole of the plants are sprinkled at the end of an experiment.

Condition of plants.

Besides the inherent variability in reaction of individual plants, already discussed, another quite appreciable cause of variation in the number of lesions resulting from inoculation was found to be the actual "condition" of the plants. The frequent watering of small pots necessary in warm weather leached nitrates from the soil, and after some time, in the absence of manuring, plants gradually came almost to a standstill. This was not so much evidenced in the early stages by a yellowness of the foliage, as by a cessation of growth in area of the leaves and a somewhat "harder" appearance of the plants. Possibly nitrogen deficiency was not the full explanation in every case, and in some instances of poorer growth the compactness of the soil in the pot may have been another important factor. At all events, plants in this condition were noticed to produce considerably fewer lesions per unit area of leaf than plants in vigorous growth.

As an example, a batch of 150 *N. glutinosa* plants, uniform when they were potted, was being raised in warm weather, and as usual in the

absence of manurial dressings a number began to come to a standstill. After some 2 or 3 weeks ten of the harder and smaller plants were selected, also ten good average plants, and ten larger and better plants from the same batch which had been trimmed to five leaves 5 days before. All were inoculated with 1 : 100 and 1 : 1000 tobacco mosaic on opposite half-leaves. The following are the mean numbers of lesions per plant produced by the two dilutions:

	1 : 100	1 : 1000	Mean diff.
10 small, rather hard, plants	20.0 \pm 2.4	6.3 \pm 1.1	13.7 \pm 1.8
10 good average plants	129.7 \pm 13.1	38.8 \pm 5.9	90.9 \pm 10.7
10 larger and better plants	155.9 \pm 16.1	48.4 \pm 9.7	107.5 \pm 7.8

When allowance is made for the different areas of the leaves of each group of plants the figures become, for the 1 : 100 virus, 57, 162 and 163 lesions per 100 sq. cm. of leaf surface respectively. It is evident, therefore, that the poor condition of the first group of plants has made them considerably less sensitive to infection by the virus. There is no significant difference between the sensitivity of the other two groups which were in good condition when inoculated.

(As regards suitability for detecting the differences between the two virus concentrations it is seen, from the third column above, that the small hard plants have given results with very nearly as high a degree of significance as the better plants. This illustrates once more the value of the half-leaf method in comparative work. It is also worth noting that trimming the plants a few days beforehand has reduced the variation and increased the degree of significance.)

In order to exclude the possibility that in the above case the plants picked out might have been of low natural sensitivity, so that the effect might not necessarily have been one of nutrition, the following further experiment was done. Out of another batch of plants allowed to go beyond the usual time without nitrogenous manuring, twenty "stand-still" plants were chosen and arranged in groups of five on a bench, so that there was no perceptible difference between the groups. Alternate rows of five were then watered with ammonium phosphate. There was a remarkable response, the treated plants resuming growth and getting big green leaves, while the untreated ones remained comparatively small and yellowish. The plants were later trimmed to five leaves and inoculated with 1 : 100 and 1 : 1000 tobacco mosaic virus on opposite sides of the same leaves. Counts of the lesions produced (mean no. per plant) were as follows:

	1 : 100	1 : 1000	Mean diff.
10 small, rather hard, plants	23.7 \pm 3.7	8.8 \pm 2.7	14.9 \pm 2.5
10 good, manured, plants	179.9 \pm 26.4	79.5 \pm 14.6	100.4 \pm 16.3

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The leaf area of the manured plants had become more than double that of the unmanured, and when a correction for the difference in area was applied the results for the 1 : 100 virus became 44 lesions per 100 sq. cm. for the unmanured and 156 lesions per 100 sq. cm. for the manured plants.

It is evident from both these experiments that if, from lack of mineral nutrients, plants are not in good healthy condition, they are considerably less sensitive to the virus. It was for this reason that occasional watering with soluble manures to promote the best possible growth was made a regular practice. The use of 5-in. pots instead of 4-in. with a better potting soil than was available to us, would probably have rendered this unnecessary.

It is not only mineral nutrition, but also other factors more difficult to define at present, which may affect the sensitivity to the virus. For example, it was found that in summer time tobacco plants grown in the greenhouse did not form many primary lesions when inoculated with samples of tomato spotted wilt virus which were considered to be quite concentrated. If the tobacco plants were removed for about 3 days to complete shade in a room lighted only by a window they became somewhat "softer" in growth, and many more primary lesions were formed on inoculation with spotted wilt virus. Naturally a number of factors are changed by such treatment. Temperature, amount of carbohydrate in the leaf, and condition of the cell walls would all be different.

An experiment was devised to prove by means of the half-leaf method that this was a real effect. Six leaves on three tobacco plants were covered on the left-hand sides with black paper (with white paper on the outside to prevent undue absorption of heat). Six leaves on three other plants were similarly treated on the right-hand sides. After 4 days the covers were removed and both sides of the leaves were uniformly inoculated with a 1 : 10 dilution of spotted wilt virus. Counts after a week showed that there were frequently more than double the number of lesions present on the sides of the leaves which had been covered with black paper. When white paper was used instead of black there was a much smaller effect in the same direction, which in the experiment cited proved only just significant with the twelve leaves used. The figures obtained were:

1 : 10 Spotted wilt on tobacco. Mean no. of lesions per half-leaf	Covered sides	Uncovered sides	Mean diff.
Plants with black covers	272 \pm 36	108 \pm 16	164 \pm 28
Plants with white covers	118 \pm 11	84 \pm 12	34 \pm 14

The experiment was repeated using 1 : 1000 tobacco mosaic on *N. glutinosa*. In this case the plants were trimmed to three leaves, five

plants were used in each group, and the covers were on for 3 days only. Once more there was in every case a greater number of lesions formed on the sides of the leaves covered with black paper, the mean difference in favour of the covered sides being 23.4 ± 3.2 , which has again a high degree of significance. When white paper was used instead of black there was a slight effect in the same direction, but the difference was not significant with only ten plants in the experiment. The figures obtained were:

1: 1000 tobacco mosaic on <i>N. glutinosa</i> . Mean no. of lesions per plant (half-leaves)	Covered sides	Uncovered sides	Mean diff.
Plants with black covers	73.7 ± 11.1	50.3 ± 9.0	23.4 ± 3.2
Plants with white covers	38.3 ± 5.4	28.9 ± 5.2	9.4 ± 5.1

When halves of the leaves were covered *after* inoculation, either with black or with white paper, no significant effect on the *numbers* of primary lesions was produced, but, particularly in the case of tobacco mosaic on *N. glutinosa*, there was a marked effect on the *character* of the lesions under the dark paper. They were considerably more spreading than on the uncovered parts of the leaves.

Effect of sand in the inoculum.

The effect of sand in the inoculum was investigated as part of a programme for determining the importance of the leaf hairs as points of entry of the virus. Since it was desired to work not only with *N. glutinosa*, but also with tobacco and *N. glauca*, Johnson's Yellow Tobacco Mosaic was chosen as the most suitable virus, and counts of primary lesions were made after iodine treatment of the leaves (except in the case of *N. glutinosa*, where the lesions are necrotic as for ordinary tobacco mosaic).

It was found that the smooth leaves of *N. glauca* were very difficult to wet with virus, and even after considerable rubbing with the ground-glass spatula dipped in concentrated virus the inoculum did not wet the whole of the leaves unless they were fairly old. A comparatively small number of chlorotic primary lesions sometimes developed from such attempts at inoculation, but on tobacco and *N. glutinosa* a virus of the same concentration invariably gave lesions far too numerous and crowded to count satisfactorily.

The effect of sand in the inoculum was therefore tried with the idea that very fine scratches might be made on the epidermal cells of *N. glauca*, which might be comparable in severity, or rather lack of severity, to the breaking of hairs on tobacco and *N. glutinosa* leaves. Twenty-five per cent. by weight of quartz river sand, ground in a mortar to pass through a 120-mesh sieve, was added to the inoculum, and this was tried

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on tobacco and *N. glutinosa*, as well as on *N. glauca*. The inoculum was washed off with water immediately after inoculation. Some results typical of many are presented in Table VI.

Table VI.

Showing the numbers of primary lesions resulting from inoculation of single leaves of N. glauca, N. tabacum and N. glutinosa with 1 : 10,000 tobacco mosaic, with and without sand, on opposite half leaves.

N. glauca inoculated with 1 : 10,000 yellow tobacco mosaic.

No sand	v.	sand	Sand	v.	no sand
1		12	25		0
5		35	20		1
0		29	50		1
0		21	15		0
2		35	37		0
<hr/>			<hr/>		
8		132	147		2
			132		8
			<hr/>		<hr/>
			279		10
			<hr/>		<hr/>

N. glutinosa inoculated with 1 : 10,000 yellow tobacco mosaic.

No sand	v.	sand	Sand	v.	no sand
13		53	15		8
2		12	11		4
1		14	12		2
0		13	19		2
1		14	17		4
<hr/>			<hr/>		
17		106	74		20
			106		17
			<hr/>		<hr/>
			180		37
			<hr/>		<hr/>

N. tabacum inoculated with 1 : 10,000 yellow tobacco mosaic.

Lesions too thick for proper counting on side inoculated with sand in the inoculum, one younger leaf where lesions not so crowded and coalesced gave:

Sand	v.	No sand
115		32

It is seen that the presence of fine sand enormously increased the number of successful primary infections on the smooth leaves of *N. glauca*. The infections on the sides of the leaf inoculated with no sand in the inoculum were due to fairly hard rubbing with the ground-glass spatula. When the leaves were merely wiped with muslin dipped in virus no infections resulted at all. The presence of sand also considerably increased the number of infections on the hair-covered leaves of tobacco and *N. glutinosa*.

Sand has been used in aiding inoculation with virus diseases by several workers. For example, Fajardo (1930) found that the most satisfactory method of obtaining a high percentage of successful trans-

missions of bean mosaic was by rubbing the surfaces of the leaves with muslin dipped in inoculum containing sand. So far it has not been possible to form a definite picture of exactly what occurred when this was done. With the aid of the iodine treatment of leaves inoculated with yellow tobacco mosaic, however, a very definite picture of what takes place is obtained (Plate X, fig. 4). It is worth noting here that the successful infections do not result from coarse scratches visible to the naked eye. These usually heal up with no infection along their length, and it is for this reason that a very fine ground and sieved sand was used in the above experiments.

Standard method described.

From the results of the foregoing experiments the following standard method was adopted for use in quantitative work such as that described in the latter part of this paper.

Attention was paid to raising good batches of test plants (tobacco and *N. glutinosa*), the seed for any one experiment being derived from a single self-fertilised mother plant, seedlings being grown under the best conditions and all being treated alike as regards transplanting and watering with soluble manures. A few days before being used for an experiment the plants were sorted out into the groups required by taking first the largest and putting one in each group, then the next largest, and so on until the groups were complete and as even as possible to the eye. In the case of *N. glutinosa* the plants were then trimmed to five leaves, and stood for 3–5 days before inoculation.

Inoculation was done with the elongated glass spatula, the flattened end being about $1\frac{1}{4}$ in. long to fit comfortably across half the leaf blade of *N. glutinosa*. The spatula was dipped into the virus under test so as to lift up plenty of liquid to cover the half-leaf, and was drawn gently but firmly over one-half of the leaf from stem end to tip, the leaf being supported on the hand covered with a fresh square of newspaper. In the earlier part of the work two rubs was the standard, there being no dip into the virus in between, but in later work a single rub was used. If the virus was more concentrated than 1 : 10, after inoculating the five half-leaves on a plant in this way, the virus was immediately washed off with a jet of water from a wash-bottle. All this could be done without wetting the opposite half-leaves, which were only inoculated with the other virus under test when the whole group of five or ten plants had been inoculated with the first virus.

In the case of spotted wilt inoculations on tobacco, owing to the larger

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size of the leaves the spatula was usually drawn down at the same angle as the lateral veins, once between each vein until the leaf was covered. In many cases, however, when spotted wilt inoculations had to be carried out quickly, the muslin method was used.

(2) *The comparison of virus concentrations from different sources.*

The most obvious application of the above methods is in the comparison of virus concentrations from different sources. Holmes (1929 *a*) has already pointed out that a far greater degree of accuracy is attainable with considerably fewer plants by using the primary lesions in place of systemic infection as a basis for quantitative comparisons. The following further examples of the value of the method may be given in addition to those cited by Holmes.

(a) *Virus content of recently diseased v. long-diseased plants.*

An early experiment was one carried out in April, 1930, to determine the virus content of tomato plants recently diseased with spotted wilt as compared with that of plants which had had the disease a long time. The experiment may be summarised as follows:

Six <i>N. acuminata</i> plants inoculated with	Primary lesions counted	Total
Virus from tomato plant diseased	on each of six plants	
6 days	12, 7, 13, 20, 15, 2	69
13 days	0, 0, 4, 1, 4, 0	9
26 days	0, 1, 0, 0, 0, 0	1

It is evident that, using only six *N. acuminata* plants per series, we have obtained a very much better comparison of the relative strengths of the viruses than we could have obtained had we used a much larger number of tomato plants and judged the results from systemic infections. A number of similar experiments have since been done with the greater accuracy attainable by the half-leaf method and with tobacco as the test plant. All the experiments have agreed in showing a marked fall in the concentration of spotted wilt in a diseased plant a week or two after the appearance of the first symptoms. Fuller details of these experiments will be given elsewhere.

(b) *Virus content of necrotic v. non-necrotic primary lesions.*

The primary lesions of tomato spotted wilt on tobacco are necrotic at ordinary temperatures (Plate X, fig. 3). On *N. nudicaulis*, on the other hand, the lesions are at first only chlorotic; gradually enlarging pallid areas indicate the points of infection, and no collapse of the tissue occurs until a late stage. It seemed of interest to compare the concentration of virus in each type of lesion.

Plants of tobacco and *N. nudicaulis* were available which had been inoculated 9 days previously with spotted wilt, and which showed numerous primary lesions. 200 cork-borer discs (5 mm. diameter) were taken from the necrotic tissue in the centre of the primary lesions on the tobacco leaves, and 200 discs were taken from the pallid, but still turgid tissue in primary infection sites on the *N. nudicaulis* leaves. Each sample was immediately ground up in 5 ml. of distilled water, and the two were inoculated on opposite half-leaves of fifteen tobacco plants. The half-leaves inoculated with the tobacco inoculum yielded only seven lesions, whereas the opposite half-leaves inoculated with the *N. nudicaulis* inoculum yielded 2619 lesions.

Other experiments showed that the spotted wilt virus could readily be detected in the green tissue immediately surrounding the margins of the necrotic lesions on tobacco, and it is most likely that the seven lesions obtained above were from virus in small bits of green tissue adhering to some of the samples. The early necrosis of infected cells leaves the virus in dead tissue, where it probably loses its virulence in a few hours. This forms a contrast to the case of tobacco mosaic on *N. glutinosa*, where there is very little multiplication of the virus in the green cells, but where the virus, owing to its resistant character, remains and can be detected in the necrotic tissue. Comparisons were also made between the multiplication of the spotted wilt virus in the primary lesions on *N. nudicaulis* leaves and its multiplication in the systemically infected leaves of tomato plants, as well as a number of other experiments of similar type.

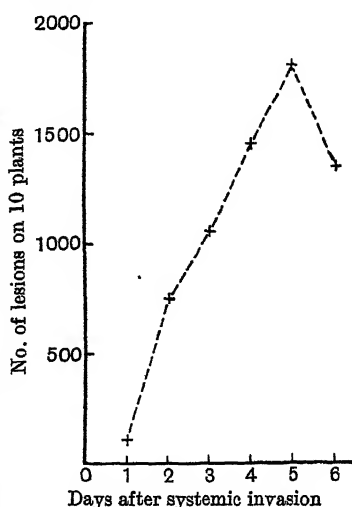
(c) *Increase of virus in the tops of infected tomato seedlings.*

A box of sixty tomato seedlings was inoculated when the plants were at the two-leaf stage (excluding cotyledons). The first plants showed systemic symptoms (bronzing) 7 days later, and each succeeding day further plants developed the disease. Each plant was marked with the date of appearance of symptoms, so that on the thirteenth day after inoculation plants were available in which the virus had developed in the top leaves for 1, 2, . . . , 6 days. Inoculum was obtained from each day group by grinding up two whole seedlings from above the cotyledons with five times their weight of water. The inoculations were done on half-leaves of ten tobacco plants for each virus, being paired against virus from the preceding day group on five plants, and against virus from the following day group on the other five.

The results are plotted in Text-fig. 6. It is seen that over the range investigated there has been a very nearly regular increase in the amount

of virus up to the fifth day, when the fall in concentration appears to have set in. This experiment was not well enough controlled to determine definitely the form of the curve of increase, but it is presented as an illustration of the type of problem that can be attempted by this method. There are a number of points of theoretical interest attaching to the more precise determination of this curve, and there is little doubt that it could be plotted also from the increase of virus in the primary lesions.

In the examples given above arbitrary figures have been recorded which show conclusively in each case that one sample of virus was much stronger than another, but no attempt has been made to say how much stronger. It is, of course, impossible to say from these figures alone how much stronger one virus was than the other. If a number of dilutions of the stronger virus had been made and one found which gave the same number of lesions as the weaker, there would then have been a method of stating the relative strength of the two viruses. It is probable, however, that further work on the dilution curve will later enable comparative strengths to be determined directly from a graph.



Text-fig. 6. Increase of virus in the systemically infected leaves of tomato seedlings inoculated with spotted wilt. Ordinates, number of primary lesions developing on half-leaves of ten tobacco plants; abscissae, days after bronzing first visible to the eye.

(3) *The plotting of curves illustrating the effect of physical and chemical agents on the virus.*

Applications of the method in the plotting of thermal death-time curves, and of curves showing the effect of chemicals on the viruses, will be presented in a separate paper. Here only one example will be given, namely, the plotting of ageing curves.

Ageing curves.

A preliminary experiment to determine changes in virulence with time of the expressed viruses of tobacco mosaic and tomato spotted wilt

was carried out by the group of ten plants method, using *N. glutinosa* for both (see Plate X, figs. 1 and 2 for illustrations of the primary lesions). Inoculations were begun immediately after expressing the viruses. One person carried out all the inoculations with tobacco mosaic and another the inoculations with tomato spotted wilt. Inoculation of each group of ten plants took nearly 8 min. Inoculation of the first five groups was done at 10-min. intervals; thereafter inoculations were done at intervals of half an hour until the virus was 3 hours old, and two further inoculations at intervals of 1 hour closed the experiment. The results are presented graphically in Text-fig. 4.

The disadvantages of the group method are evident in these curves. The points are considerably scattered and the differences between many are not statistically significant, but general tendencies are plain enough. In both viruses there was apparently an initial increase in the concentration of infective particles soon after the juice was expressed. In the tobacco mosaic this increase took some 3 hours to reach the maximum, after which there are indications that the virus concentration was remaining fairly steady. With the tomato spotted wilt, however, the increase was more rapid than in the tobacco mosaic, and in half an hour the concentration was already commencing to fall. In 2 hours the virus was non-infective on *N. glutinosa*.

No further tests were done with tobacco mosaic. Holmes (1928) has presented some figures showing changes in concentration in this virus over a period of some months. A number of further tests were done with tomato spotted wilt however, and in some of them evidence was obtained of an initial rise in concentration soon after extraction, but in others no rise was observed. The conditions determining the occurrence or otherwise of this initial increase in concentration have not been further investigated. Starting from the maximum point, however, the curve showing loss of virulence with time in the case of the rapidly ageing tomato spotted wilt virus is of a definite type, illustrated for two experiments (in which no initial rise was observed) in Text-fig. 5.

The points in this figure have been plotted from the experimental data shown in Table VII. The sap from diseased plants was expressed, strained through muslin, and divided into eight samples in Exp. Q 49 (and into ten samples in Exp. Q 80). The first sample was then immediately inoculated by the muslin method on to half-leaves of groups of tobacco plants previously prepared. These plants were so arranged that each time sample was compared on opposite half-leaves with the immediately preceding and immediately following time sample, in one case being

inoculated on the left halves of the leaves and in the other case on the right halves of the leaves. Immediately after inoculation of each group of plants the virus was washed off the leaves with a stream of tap water. The times given are the times when inoculation of each sample commenced. Inoculation of each group took about 8 min., so that if an average time for inoculation of a sample was taken the time points would be shifted 4 min. to the right. This, however, would not alter the form of the curve.

Table VII.

"Ageing," or loss of virulence with time, of the virus in the expressed juice of tomato plants diseased with spotted wilt.

Exp. Q 49.			Exp. Q 80.		
Temp. 22° C. (72° F.). Twenty tobacco plants inoculated on two half-leaves from each sample			Temp. 19° C. (66° F.). Ten tobacco plants inoculated on two half-leaves from each sample		
Time in min.	No. of lesions	k	Time in min.	No. of lesions	k
0	3135	—	0	3395	—
10	1380	0.356	20	1601	0.163
20	573	0.369	40	1045	0.128
30	294	0.342	60	672	0.117
40	68	0.415	90	231	0.129
50	22	0.431	120	107	0.125
90	14	0.261	180	23	0.121
180	0	—	240	5	0.118
			300	1	0.118
			360	0	—
Mean		0.362	Mean of 7		0.122

The values of k given in Table VII have been calculated from the equation $k = \frac{1}{t} \log \frac{a}{a-x}$, where a is the number of lesions formed by the fresh virus, and $a-x$ is the number formed at the successive time intervals. When account is taken of all the experimental difficulties, the agreement between the values of k in each experiment may be considered very satisfactory. The first value of k in Exp. Q 80 appears to be abnormally high, and it has been disregarded in taking the mean. The curves drawn in Text-fig. 6 are the theoretical curves obtained when constant values of k of 0.362 and 0.122 are used in the logarithmic equation. The agreement between the experimental points and these curves is evident, and, of course, when plotted on a logarithmic scale the values lie closely distributed about a straight line. This logarithmic type of curve, corresponding to that of a unimolecular chemical reaction, suggests that the process of "ageing," or loss of virulence with time of the expressed virus of tomato spotted wilt, is possibly a simple chemical

reaction, perhaps of the nature of an oxidation. Text-fig. 5 also illustrates the different speeds at which the process takes place at different temperatures. This will be further discussed in a following paper on thermal death-time curves, for in the case of tomato spotted wilt it is impossible to say where the "ageing" effect ends and the thermal effect begins, if, indeed, the latter, in its usual sense, begins at all.

DISCUSSION.

From the work presented here it is evident that, provided statistical methods are employed, the use of the primary lesions will yield considerably more accurate data on the properties of certain plant viruses than could ever be hoped for from the older method, based on systemic infection and the very variable needle inoculation. The case chosen for illustration in this paper, namely, the ageing curve for the virus of tomato spotted wilt, brings this out particularly well. In the first place spotted wilt is a virus which it is almost impossible to inoculate with a needle, and rubbing of the leaf surface is the only efficient method of mechanical transmission (Bald and Samuel, 1931). Also the virus dies so rapidly after extraction that numbers of individual plants, which would at all compare with the numbers of primary lesions which may be observed by the rubbing method, could never be inoculated in the time available. Using the primary lesion method curves may be drawn with an accuracy not only sufficient to illustrate the general course of the phenomenon known as ageing, and to place it definitely among reactions of the logarithmic type, but also to determine within reasonably narrow limits the influence of such factors as temperature, etc., on its speed.

The objection may be raised that there are only some two or three viruses which are known to form necrotic primary lesions suitable for quantitative work of this type. It is undoubtedly true that there are many viruses which it will not be possible to investigate in this way, but it seems probable that a number more than are known at present will be found to form suitable primary lesions on some plants. Henderson and Wingard (1931) have recently given the results of thermal treatments on tobacco ring-spot virus in terms of primary lesions. Samuel (1931) used the primary lesions formed by one constituent of potato rugose mosaic on tobacco and *N. rustica* in some experiments on inoculating methods. Also E. M. Johnson (1930) and Samuel (1931) both mention primary lesions formed by cucumber mosaic on the leaves of sugar beet.

Even if no visible primary lesions are formed, however, these studies should make for improved methods of quantitative work when systemic

infection is used as the basis. There can be little doubt that the course of infection in such cases is substantially the same, the difference being only that the virus causes no necrosis, chlorosis, interference with starch metabolism, or other changes by which its presence in local sites may be detected. By way of example, it is probable that at least part of the dilution curve or the thermal death-time curve for bean mosaic could be drawn if the inoculation technique was standardised by rubbing a certain area of leaf each time with virus containing a definite amount of sand of a definite fineness. This would merely be equivalent to the way in which Holmes (1928) drew part of the dilution curve for tobacco mosaic using the standardised needle-prick method of inoculation on trays of tobacco seedlings. Such large numbers of plants are required for this method, however, that the result would often not be worth the work entailed. If some other species of plant could be found on which bean mosaic formed visible primary lesions the work would at once be made both shorter and more accurate.

There is, therefore, an appreciable field of work opened up by the primary lesion method in the more accurate investigation of plant virus properties. In this paper the drawing of conclusions from the types of curve obtained in dilution and ageing experiments has purposely been avoided. It is obvious that more detailed investigations will need to be carried out on all the points raised. Moreover, it is not to be expected that the details of technique which have been outlined will prove the most satisfactory in the end. Improvements will undoubtedly result from further work by different investigators in the future.

SUMMARY.

The precautions which should be observed in practical application of Holmes's local lesion method in quantitative work with tobacco mosaic have been examined. The aim has been to obtain results with the greatest statistical significance consistent with the use of reasonably small numbers of plants. The adoption of a standard method of inoculation, and the comparison of viruses on opposite halves of the same leaves, were found to be the most important points to observe, and a method of work has been outlined.

Tomato spotted wilt forms necrotic primary lesions on the leaves of tobacco of a character suitable for quantitative work, and a number of the results obtained with tobacco mosaic have been checked with this virus.

Applications of the method are discussed. It is shown that rapid comparison of virus concentrations from different sources should be

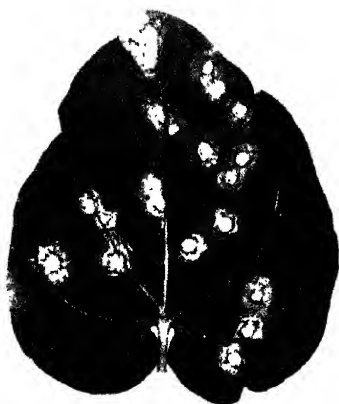


Fig. 1.



Fig. 2.

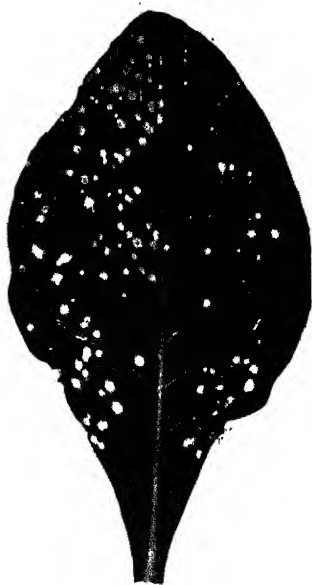


Fig. 3.



Fig. 4.

SAMUEL AND BALD.—ON THE USE OF THE PRIMARY LESIONS IN QUANTITATIVE WORK
WITH TWO PLANT VIRUSES (pp. 70-99).

possible after further work has been done on dilution curves. An illustration is also given of the application of the method in plotting the curve of "ageing," or change in virulence with time, of the expressed virus of tomato spotted wilt. This curve was found to be of the logarithmic type.

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EXPLANATION OF PLATE X.

- Fig. 1. Primary lesions of tomato spotted wilt on *N. glutinosa* leaf, 9 days after inoculation.
Fig. 2. Primary lesions of tobacco mosaic on *N. glutinosa* leaf, 9 days after inoculation.
Fig. 3. Primary lesions of tomato spotted wilt on leaf of Blue Pryor tobacco, 5 days after inoculation.
Fig. 4. Effect of fine sand in the inoculum in increasing the number of successful points of entry of the virus of yellow tobacco mosaic on the smooth leaves of *N. glauca*. Both sides of the leaves inoculated with 1:10,000 yellow tobacco mosaic by ground-glass spatula; left sides with no sand in the inoculum, right sides with inoculum containing sand. Leaves treated by the iodine method to show up the primary lesions.

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THE PHYSIOLOGY OF VIRUS DISEASES IN PLANTS

IV. THE NATURE OF THE VIRUS AGENT OF AUCUBA OR YELLOW MOSAIC OF TOMATO

By JOHN CALDWELL.

(*Department of Mycology, Rothamsted Experimental Station.*)

(With 1 Text-figure.)

DURING the past few years, as the symptomatology of plant virus diseases has been increasingly clarified, more attention has been directed to the study of the nature of the virus. Recently, it has been shown by Barnard that the probable agent of one of the animal diseases hitherto considered as a virus disease can be seen under suitable conditions. Elford (4) has, at the same time, shown by improved filtration methods that the size of the agent would correspond with Barnard's observations. Even yet, however, most of the virus disease agents appear to be much smaller than that investigated by Barnard and Elford (1).

One of the main difficulties of technique which so far has confronted the workers on the nature of the agent has been the impossibility of culturing the virus *in vitro*. Kendall has recently developed a technique by which it is possible to induce the formation of a filter-passing stage in bacteria, and this may prove to be of value in virus work. At present, however, the only reported case of multiplication of a plant virus in a dead culture medium—that of tobacco mosaic—has not been confirmed (7).

That the virus is an organism is becoming more and more widely believed, at least as a working hypothesis, and the demonstration of the presence of particles in serum from a diseased animal rather supports this belief. How far, however, the particulate nature of the virus may be due not to the virus itself but to the aggregation round it of cell constituents, is a point not yet settled. It has been shown for many virus diseases that one characteristic symptom is the formation of precipitation products in the cytoplasm. It is easily shown, also, that the virus is intimately associated with the normal cell contents, since it is readily precipitated with the cell proteins. At the same time, the observation that some plant

viruses apparently increase in virulence if the macerated juice be kept for some days is not necessarily evidence of disintegration of masses of virus as Olitsky⁽⁷⁾ has suggested. It may be due to the breakdown of cellular material and the release of particles in that way. Later in this paper it is shown that there is little evidence for supposing that the virus of aucuba or yellow mosaic of tomato is normally aggregated into masses either mechanically or by adsorption round a large protein body.

In work with animal viruses and, until recently, with plant viruses, the study of the particulate nature of the agent and the nature of the particle, if such existed, was complicated by the fact that the only method of assessing the presence of the agent was by inoculation of small amounts into healthy host animals or plants. This method does not readily allow of work with large volumes of liquid, and sampling at high dilutions is rendered more than usually difficult. As a consequence, little information is available as to the number of virus bodies required to induce infection, except that it appears to be small, or the number of virus bodies present in an infected cell, except that it appears to be very large.

A recent paper of Holmes⁽⁶⁾ has directed attention to the fact that one type of experiment would furnish useful data on this problem. This could be carried out with a "standard" virus on a plant, and the conditions are such that unusually large numbers of plants need not be used. Holmes showed that when tobacco mosaic juice was rubbed on to the leaves of *N. glutinosa* and numerous other of the Solanaceae, lesions appeared on the rubbed leaves. The earlier and now obsolete technique which had insisted on the perforation of the lamina of the infected leaf had, by the extensive damage done, largely prevented the recognition of the local lesions.

As it is possible to bring about the infection of the plant merely by rubbing the leaves sufficiently roughly to ensure that a number of the hairs are broken without at the same time damaging the mesophyll tissues the local effect of inoculation may readily be examined. It has been found, for instance, with aucuba mosaic in tobacco, that the first symptoms which develop are local lesions on the rubbed leaves, and these precede the more usual symptom of chlorosis of the developing leaves.

The writer has shown in an earlier paper⁽³⁾ that the aucuba or yellow mosaic of tomato has a similar effect on *N. glutinosa* and also on tobacco. The interesting point is that under the conditions in our experimental glasshouses the disease does not, in *N. glutinosa*, at any time become systemic, but that the symptoms and indeed the agent are localised on the rubbed leaves. At the same time the writer has shown

that the appearance of symptoms on the *N. glutinosa* depends on the damaging of leaf tissue, and that while it is not necessary to perforate the lamina as had regularly been done heretofore, it was necessary, to ensure infection, to break the hairs on either side of the leaves. Holmes has pointed out that the number of necrotic lesions which occurred on the leaves was some index of the dilution of the sample of infectious juice, that is of the amount of virus present in the inoculum. He apparently inoculated the leaves by rubbing with a piece of cotton-wool soaked in the juice, and the method does not allow of very careful analysis of the data. It was evident, however, that if a suitable method were devised one had here a technique for demonstrating that the virus agent was particulate in nature and also of assessing the probable numbers of the particles present in any given inoculum.

If it could be shown that the number of necrotic areas bore a definite and constant relation to the amount of dilution then the supposition would be that the agent was particulate. If it could, further, be shown that the number of necrotic areas could not be increased in any given dilution by any treatment calculated to break down aggregates of particles or to free particles of the agent from adsorbing materials, then the presumption would be that the virus particles were separate entities which could exist alone. It would be shown, therefore, not only that one particle was sufficient to induce the formation of the given symptom but also that the number of such particles could be calculated to a fair degree of accuracy.

By the use of such a plant as *N. glutinosa*—tobacco was used to check the observations—it was possible to carry out accurate experiments on a sufficiently large scale—since each leaf acted as a separate organism, and the total number of plants used was comparatively small. It has been pointed out in a previous paper in this series that the symptoms can and do appear on leaves of *N. glutinosa* which have actually been detached from the plant(s), and that the agent does not move about the plant. The top of a plant infected below is quite normal and, in fact, the virus does not travel across the lamina of the rubbed leaf on to the uninoculated portions.

METHODS AND MATERIALS.

The infectious juice was prepared by macerating in a mortar a number of leaves of tomato or of tobacco from plants showing good, typical symptoms of aucuba mosaic. To the crushed pulp was added distilled water in the proportion of 2 c.c. of water to 1 gm. of pulp. This

was thoroughly mixed with the tissue and the whole left for 24 hours on the laboratory bench. Thereafter, the pulp was put into muslin and as much as possible of the liquid expressed. This liquid was then passed through a fluted filter paper impregnated with fuller's earth when the filtrate was clear and brown coloured. This liquid was considered as the source of material, and dilutions were made from this as stock. Some of this material was freshly prepared for each experiment.

In the earlier experiments, which were of a preliminary nature, the stock material was diluted by the addition of 10 to 90 c.c. of water giving 1/10 dilution; of that 10 c.c. were added to 90 c.c. of water giving 1/100 dilution and so on. After each dilution the liquid was mixed as thoroughly as possible by prolonged shaking.

The diluted material was put on to the experimental plants. Plants of *N. glutinosa* with some five to ten leaves were taken, and on to each leaf was rubbed 0.1 c.c. of inoculum. This inoculum was carefully spread with the top of the index finger over the whole surface of the leaf, an attempt being made to break the hairs on the adaxial side without, so far as possible, damaging the tissues of the mesophyll. It has already been shown that rupture of the living cells must be made before infection can take place and the necrotic lesion, the only symptom, be formed (3).

EXPERIMENTS ON SERIAL DILUTION.

In the first experiment, the number of leaves used was five in each group and each was rubbed with 0.1 c.c. of inoculum as described. The top of each plant was removed, following the practice of Holmes, and an examination of the plants was made a week after treatment. The results are indicated in Table I.

Table I.

Number of spots on ten treated leaves of N. glutinosa.

Dilution	1/100	1/1000	1/10,000
No. of spots (total)	146	14	2

In another experiment the results were similar. In this instance, the number of leaves used was ten on each plant, and total numbers obtained for each dilution are shown in Table II.

Table II.

Experiment B. Number of spots on ten treated leaves of N. glutinosa and different dilution.

Dilution	1×10^{-1}	1×10^{-2}	1×10^{-3}	1×10^{-4}	1×10^{-5}
No. of spots (total)	449	562	68	8	1

In this experiment at dilutions 1×10^{-1} and 1×10^{-2} the number of broken hairs seems to have been a factor of much importance, as evidenced by the abnormal results obtained, but in the rest of the series, as in the previous experiment, the results are very significant.

This result has been confirmed on many occasions, and there seems to be no doubt that the number of spots obtained bears a direct relation to the dilution under the conditions of this experiment. The relationship breaks down at high concentrations where apparently there are not enough broken hairs to admit of the entry of the agents present and where actually more agents enter at a broken cell than is necessary as a minimal infection dose.

One point has to be borne in mind and that is that the hairs are not so easily broken on the slightly immature leaves, indeed the ease with which hairs are broken tends to vary from leaf to leaf with various environmental or physiological conditions. The distribution of the spots on the leaves in one dilution is shown below:

Leaf from top	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th
No. of spots	5	21	24	28	49	22	34	45	50	28	28

It was generally found that the younger leaves at the top of the plant were consistently less well-spotted than those lower down, though in the later experiments, with more experience, the distribution of spots became more regular and the method much more accurate. The reason for the fewness of the spots on the upper leaves seems to be the less brittle nature of the hairs on these leaves. It has been clearly demonstrated that rupture of the cells is necessary for infection, and the rupture takes place usually at the hair base. If the hair base be fairly tough and can resist the not very great pressure of the finger, clearly the chance of infection is reduced. Another point which is of interest is that the necrotic areas on the upper leaves are consistently smaller than are those on the lower. It has been suggested before that the amount of multiplication which takes place in these areas is small, and that the effect of the agent is to kill a few cells round the point of inoculation (cf. (2)). When the leaves are mature and the mesophyll cells of the full size are separated by fairly large intercellular spaces, the area affected by the collapse of a given number is much larger than it would be if the same number of cells were more compact and not so well developed. Observations of this kind must necessarily be borne in mind in any consideration of the experiments.

Yet another point which is of great importance in these experiments is that the amount of pressure which has to be applied is restricted

within quite narrow limits. The hairs of the adaxial side must be broken fairly evenly over the surface to allow of the entry of all the virus particles into ruptured cells. At the same time it has been shown that excessive rupture of the mesophyll cells, which are the cells responsible for the exhibition of symptoms, will prevent the development of any symptoms. The writer has often found that light rubbing on one half of a leaf induced symptoms on that side whilst heavy rubbing on the other half induced the appearance of few or no symptoms.

So far as our experience here is concerned, the difference between leaves is great. On some plants rubbing of a given inoculum induces many necrotic spots per leaf, while on others the same inoculum similarly treated induces but few. Environmental conditions also play an important part in determining whether the hairs on the leaf will be easily broken off or not.

MORE DETAILED EXPERIMENTS ON DILUTION.

The results of the experiments involving dilutions of 1/10, 1/1000, 1/10,000, etc., were so satisfactory that it seemed desirable to decrease the interval between the dilutions. That is to say, the dilutions were made so that each in the series was twice as dilute as the one preceding it. Such a series is common in bacteriological technique and affords a satisfactory method of counting the bacterial numbers. The same general method was employed as before. Plants of *N. glutinosa* were selected which were just on the point of flowering. Normally, under our conditions, plants at this stage have ten or twelve well-developed leaves and have perhaps sixteen leaves in all. The lower seven or so leaves were removed as was also the whole top of the plant, together with the uppermost three leaves. As a consequence there remained five or six well-formed, uniform leaves mature and normal in type which afforded, as evidenced by a large body of data, the best material for the development of necrotic lesions. In these leaves there are more consistent numbers of spots than in leaves chosen on the whole plant, where, as we have seen, the upper and lowermost leaves have fewer spots than have those in the middle portion. Even in the leaves of the middle portion of the plant, however, it is not possible to ensure that the rubbing be sufficiently hard to break a large enough number of hairs to ensure that broken hairs be not a limiting factor and be not so rough as to cause damage to the soft mesophyll tissues which are very easily crushed even in the toughest leaves. It does not seem to be possible to overcome this difficulty

completely, and attempts at removing the hairs by "shaving" with a sharp razor have been even less successful than the method outlined.

In these experiments, as has been pointed out, the dilution was in each case twice that of the previous and, consequently, in the light of the factors which have been outlined above, very close agreement was hardly expected. Actually, the results obtained were very good and approximated to the theoretical.

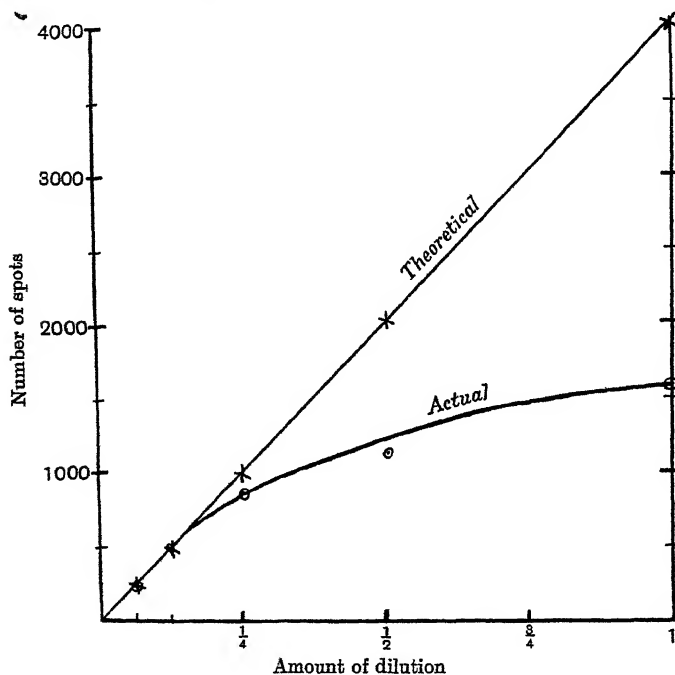


Fig. 1. Graph showing the relationship between the theoretical and observed number of spots with certain dilutions.

In most experiments it was clear that, at the higher concentrations of juice, the number of broken hairs on the leaf surface became a limiting factor. This was well borne out in an experiment in which the undiluted juice was used, the second inoculation was that juice diluted $1/2$, the third $1/4$ and so on. The figures obtained were 1610, 1090, 882, 540 and 245 as totals of the numbers of spots on ten leaves rubbed with juice, undiluted, diluted $1/2$, $1/4$, $1/8$, $1/16$ respectively. If one assumes that the lowest figure was correct at 250 the series would have been, 250, 500, 1000, 2000 and 4000 for the required dilutions. It is clear that at the

higher concentrations, some factor has operated, which was not the amount of virus present. The higher the concentration, *i.e.* the greater the amount of virus in the inoculum, the greater is the discrepancy between the observed and the theoretical results. It would appear, therefore, that the factor which is mainly concerned with the discrepancy lies in the tissues rather than in the inoculum. It seems reasonable to assume that the number of hairs which were broken was insufficient to make available a large enough number of points of inoculation to render effective all the virus. Results of this experiment are set out in Fig. 1, where it will be seen that the discrepancy between the theoretical and the observed results is much greater as the numbers increase in size.

The preliminary experiment had shown that dilutions of about 1/50 furnished useful material for this work and did not induce the formation of too large numbers of spots. Here, again, there was great individual variation, and it was not possible accurately to foretell the strength of a virus inoculum from the intensity of symptoms on the plant from which it was taken. Actually, the less well-grown plants in the glasshouse during November had rather higher virus content than those grown in the spring and early summer.

The results of some of the experiments are given in Table III.

Table III.

Total numbers of necrotic spots on ten leaves of N. glutinosa.
Inoculum 0.1 c.c. Aucuba mosaic juice at given dilution.

Date								
Feb. 15th:								
Dilutions	1/250	1/500	1/1000	1/2000	1/4000			
No. of spots	365	207	105	44	36			
Apr. 22nd:								
Dilutions	1/250	1/500	1/1000	1/2000	1/4000			
No. of spots	296	113	49	26	—			
Aug. 12th:								
Dilutions	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400
No. of spots	530	300	230	90	40	50	11	3

From these data, which are only a few of the many experiments carried out, there is strong presumptive evidence, all the above-noted considerations being taken into account, that there is a direct and simple relationship between the dilution and the number of spots when equal amounts are used on each leaf. The inference drawn is that the virus agent is apparently particulate in nature, and that each particle, or group of particles, is able to induce the appearance of a necrotic spot on the leaf of *N. glutinosa* if entry into a cell be effected.

THE DISINTEGRATION OF AGGREGATIONS OF PARTICLES.

In this laboratory no evidence has been obtained to show that agitation of the virus inoculum has the effect of increasing the number of particles present, after care has been taken to ensure that the juice has been freed from cell debris. It is obvious that this precaution must be taken because masses of cytoplasm might well contain numbers of particles which would be liberated as the proteins degenerated. It has long been known that some increase does take place in juice which after maceration is allowed to stand for 24–48 hours, and it is now generally agreed that this apparent increase is due to autolysis of the tissues.

The question under consideration is, however, the disintegration of masses of virus bodies aggregated so that the mass functions as an individual particle. Two possibilities suggested themselves, (a) the virus bodies might be loosely grouped together—in which case they should be separated either by agitation or by standing, and (b) the bodies might be adsorbed in groups to a large protein molecule, in which case they would tend to separate on digestion of the protein.

The first possibility was examined first. An inoculum was prepared by macerating a quantity of tomato-leaf mosaic with twice its weight in water. This material was allowed to stand on the laboratory bench overnight. After 24 hours it was passed through a fluted filter paper impregnated with fuller's earth and the filtrate diluted to 1/50. This material was well shaken and allowed to stand for 24 hours. The first inoculation, with 0.1 c.c. rubbed on each of five leaves of two plants of *N. glutinosa*, was made 48 hours after the infected leaves had been picked.

Table IV.

Number of spots per ten leaves of N. glutinosa, inoculated with 0.1 c.c. of material after 48, 72, 96, 120 and 144 hours.

Series	Time hours	No. of spots on ten leaves	
		Exp. 1	Exp. 2
A	48	18	276
B	72	19	277
C	96	7	190
D	120	6	78
E	144	13	107

Five series of leaves, A–E, were inoculated. Each series was inoculated by rubbing in the usual way with 0.1 c.c. of inoculum 24 hours after the previous group. In the interval the inoculum has been allowed to stand in a stoppered Erlenmeyer flask on the laboratory bench, and it

was shaken at intervals. No toluol was added to reduce bacterial growth and some bacterial contamination took place. This was not very pronounced, however, and did not, apparently, greatly affect the results. The data obtained from two experiments are detailed in Table IV.

It may be seen from these results, that in these earlier experiments which are taken as representative of a group of similar experiments there is no evidence that there is any increase in the number of particles by the breakdown of groups into smaller units.

THE DIGESTION OF THE PROTEIN IN THE INOCULUM.

The other possibility to be considered was the adsorption of the virus bodies to protein molecules. This seemed not improbable in the light of the fact that protein precipitants have the effect of removing virus from infectious juices. An inoculum was prepared from tomato-leaf tissue in the usual manner. This was diluted to 1/50. Thereafter 100 c.c. were put into an Erlenmeyer flask with a cotton-wool plug. A few drops of toluol were added. This served as a control. To another 100 c.c. similarly treated was added 0.1 gm. of trypsin powder and to another a like amount of pepsin. All three were kept in an incubator at 25° C. for 24 hours. Thereafter, inoculations were made by rubbing in the usual manner 0.1 c.c. of the liquid on each of a number of leaves of *N. glutinosa*. Counts were made of the spots which developed on the leaves after a week, and the data showed that whereas there was no apparent increase or decrease in the number of spots induced by the pepsin-treated material over that induced by the control liquid, the trypsin had had the effect of reducing, in all cases very considerably, and in others actually to one or two, the number of spots found on the treated leaves. It is shown that this reduction is probably due to an adsorption of the virus to the enzyme rather than to a destruction of the virus by the enzyme. For the moment, the main interest attaches itself to the fact that destruction of the protein in no wise increases the number of the spots formed on inoculation. It therefore appears that the virus is not aggregated into masses which easily break down mechanically, nor is it adsorbed in groups on to the proteins of the plant.

THE NUMBER OF VIRUS PARTICLES PRESENT IN A JUICE.

It was thought that if certain assumptions were made in the light of the foregoing experiments, it should be possible to give an approximate idea of the number of virus particles present in any given juice. The initial concentration of juice is known; let us say that the dilution is

1/100 and that 0.1 c.c. of this juice is rubbed on the surface of a leaf of *N. glutinosa*. Let us assume further that fifty spots are formed as a consequence of inoculation. The spots are formed following the entry of the virus into a broken hair. The majority of the hairs which are broken by rubbing fracture just at the base, so, for practical purposes, it may be taken that the entry is made at a ruptured hair base. If one assumes, further, that the inoculum is evenly distributed over the surface of the leaf the number of particles which enter hair bases will be a fraction of the total number spread over the surface. This fraction will be dependent on the relation between the area of the hair bases and the surface of the leaf. Actually, in cross-section, the hairs are found to be very numerous and the hair bases occupy approximately one-third of the epidermis in a transverse section of the leaf. They are fewer over the veins and the distribution is not absolutely uniform, so for purposes of argument it is suggested that one-tenth of the leaf surface might be considered as occupied by hair bases. As has been pointed out, the ease with which hairs break varies greatly from plant to plant and from leaf to leaf, and in the same way the ease with which individual hairs on any one leaf break also probably varies considerably. It may, therefore, be necessary to make some allowance for the proportion of whole to broken hairs. To off-set this, however, there is the consideration that there is probably a tendency for more of the particles to attach themselves to hair bases than to remain on the surface of the leaf, since the bases are slightly raised above the general surface of the epidermis, and any particles being carried over the surface in a film of liquid will tend to hit the broken bases. It is suggested that it may, for purposes of this argument, be assumed that it is fair to consider that the number of particles present on a leaf surface is represented by ten times the number of the spots formed.

To revert to the supposed observation that fifty spots had been found as a consequence of rubbing 0.1 c.c. of a juice diluted 1/100, it follows, therefore, that the number of particles in the original diluted juice was

$$50 \times 10 \times 100 Y \text{ per c.c.} = 50,000 Y \text{ per c.c.,}$$

the value of Y , i.e. the proportion of virus particles which enter broken hairs on the surface of a leaf of which one-tenth of the area is occupied by hair bases, is, as we have seen, difficult of assessment but may be taken as 10, as we have suggested.

It therefore follows, if the validity of this argument be admitted, that a fair approximation to the number of particles may be arrived at by

taking the average number of spots on a given number of leaves or, alternatively, by taking the number of spots for the greatest dilution and considering the numbers obtained from them as being the maximum figure.

For example, in the experiment of November 10th, 1931, there was one spot on each of two leaves of the five. The dilution was $1/100,000$, so that the total number of particles, using the method outlined above, was $1 \times 10 \times 100,000 \times 10 = 10,000,000$ per c.c. of the original inoculum. This was made by macerating tissue with twice the weight of water, so the original tissue contained rather more than 3×10^7 particles per c.c.

THE EFFECT OF ENZYMES ON THE VIRUS AGENT.

In the experiment with trypsin and pepsin digestion outlined above it was found that whereas pepsin appeared to have no effect on the number of spots which formed after inoculation, trypsin had the effect of very much reducing the number. That this is not due to a definite destruction of the virus agent by the enzyme is indicated by the fact that heating the virus material to 70°C . for 20 min. after incubation had the effect of increasing the number of spots on subsequent inoculation to a number comparable with that formed with the control material. Both taka-diastase and malt-diastase, freshly prepared from barley, had a similar effect to trypsin of reducing the number of spots which were formed, and heating in these cases did not restore the efficiency of the inoculum. It was not possible, however, with these enzymes to destroy them, as judged by their effect on starch, without heating above 70°C . This would, at the same time, have destroyed the virus which will withstand heating to 80°C . for only a short period. At the same time, boiling the enzyme in water before treatment of the virus had the effect of rendering it innocuous. The presence of iodine in potassium iodide solution, which itself had no effect on virus numbers, was also able, with malt-diastase, to prevent the interaction of the enzyme with the virus. The possibility of the virus having a specific reaction on the tissues of the host plant was examined. It is possible to account for the reduction which occurs in these experiments with enzymes and aucuba mosaic by assuming that the enzymes react on the broken hair bases so as to prevent the entry of the virus into them. To demonstrate that this was not so, the following experiment was set up. A mixture of 0.2 gm. of trypsin powder with 20 c.c. water was made up. This was rubbed carefully over the leaves of *N. glutinosa* plants as would have been an inoculum. Thereafter, an inoculum of aucuba mosaic was also rubbed over the

treated leaves. This was further rubbed over other leaves on the same plants which had had no trypsin treatments. The amount of the inoculum was the same in both cases. The average number of spots per leaf for the trypsin-*aucuba* mosaic was 38 and for the controls 43. It would appear, therefore, that effect of these enzymes is to adsorb the virus and so inactivate it, rather than to act specifically upon it and break it down or to render impossible the entry of the particle into the plant tissue (cf. Holmes (6)).

THE MULTIPLICATION OF THE VIRUS WITHIN THE
TISSUES OF *N. GLUTINOSA*.

As has been pointed out above there is reason for supposing that a single virus particle of the *aucuba* mosaic virus will on entering a broken hair of *N. glutinosa* give rise to necrosis. From earlier experiments (3) it had appeared that the multiplication of the virus within the tissues of the *N. glutinosa* plant was not great, and in no event did the disease become systemic. The method of counting the necrotic spots induced on a leaf affords a more precise method of assessment of the amount of virus present in a tissue than does the older method of inoculation of the suspected juice into groups of healthy seedling tomatoes. The procedure adopted was to remove each spot from the leaf and a portion of the surrounding tissues with a cork borer of 5 mm. diameter. Of these discs one to five were taken and macerated in watch-glasses with the minimal amount of water 0.1–0.25 c.c. This material was rubbed on the surface of a leaf on *N. glutinosa*. As a consequence of a large number of such experiments it would appear that the number of spots which appear on the treated leaves is some 15–30 per 0.1 c.c. in the original inoculum. If one assumes that the original spot was induced by one particle and that the number of spots is indicative of one-tenth of the number of particles actually present on the leaf, then the multiplication has been of the order of 250 times. Even if a single particle were not involved in each necrotic spot but a group of particles, the same argument holds.

The multiplication would appear to be large if one had not, at the same time, examined the numbers of the virus within a disc of tissue of a leaf of a tomato plant infected with *aucuba* mosaic. When a single such disc was macerated in water and rubbed over the surface of a leaf of *N. glutinosa* the whole lamina became pitted with necrotic areas which were so close as to become confluent. Actually, counting on such leaves is very difficult, but the number of spots formed was of the order of 500. Since the number of broken hairs at this point has become a limiting

factor it is clear that the multiplication in the tomato tissue must be very much greater than in the *glutinosa*. Inoculations had not originally been made in the leaves from which the discs were taken, so that this multiplication had clearly taken place throughout the whole of the plant. If one makes the same assumptions as were made for the *glutinosa*, viz. that one particle first entered the disc under observation and there multiplied and that the number of particles on the *glutinosa* lamina was ten times the number of spots, the multiplication of the virus in the tomato tissue was of the order of 10,000 or more times.

DISCUSSION.

Considerable importance has been attached to the difficulty of breaking the hairs in the leaves on *N. glutinosa* when inoculations are being made with infectious juice. As has been pointed out no satisfactory method of inoculation has suggested itself which would overcome the difficulty of the non-breaking of hairs or the destruction of the mesophyll tissue. Another factor which has to be considered in the light of the results which have been obtained is the difficulty of obtaining comparable inocula from different plants and different tissues. For example, when an inoculum diluted 1/100 and lower is prepared in the usual way it is customary to find some 20-100 spots on each leaf rubbed with 0.1 c.c. From some plants, however, a dilution of this amount would give only one or two spots and would be unsuitable as a source of material for dilution experiments. What controls the amount of virus which is present in any given plant is not clear, but it would appear that intensity of symptoms in the host tomato plant is not an evidence of high virus content.

These and other considerations render almost impossible the obtaining of consistent results in every experiment. At the same time, many of the experiments have yielded results which can be shown to have a high degree of statistical significance. In other experiments, by reason of the operation of one or more of the factors indicated above, some of the data were not in accordance with expectation.

The fact that many of the experiments did yield results which indicate that the number of spots bears a direct relationship to the degree of dilution is taken to indicate that the virus is particulate and that one particle is able to set up a necrotic spot if effective entry is made into the tissues.

A simple experiment was set up to show in another way that the number of spots formed is an index rather of the number of particles

than of the number of spots at which entry into the leaf tissue can be effected. A large series of leaves were rubbed and it was found that the average number of spots induced by inoculation of 0.1 c.c. of a virus inoculum was 22.3.

A further set of leaves was inoculated with the same virus, and a third set was inoculated by rubbing the same amount of inoculum on one half of the lamina leaving the other half of the lamina clear. In this experiment, therefore, the same amount of inoculum was rubbed on two sets of surfaces, one having half the area of the other. If the number of spots formed bore any relation to the amount of surface rubbed, the leaves of which only one-half was rubbed, should have only half the number of spots. The leaves with the whole lamina rubbed had an average 22.7 spots, and those of which the half was rubbed 25.6 spots. It is clear, therefore, that the area rubbed is not the controlling factor in the formation of spots, so long as there is a sufficiently large number of broken hairs present to admit of the entry of a particle into each.

The experiments in which the juice was, before inoculation, treated with enzymes or coagulated to break up the aggregations of virus particles, if such existed, tend to show that the particles are not grouped together in the inoculum except in so far as masses of cell debris may contain numbers of particles. The cell debris was removed in every case in the experiments outlined above by filtration of the inoculum through fuller's earth.

In the calculation of the number of particles which is present in any given inoculum one or two assumptions had to be made. One was that the hairs broke fairly easily over the whole surface of the leaf, and that, therefore, the area of the hair bases could be considered as the area of the surface available for the effective entry of the virus particles. The actual area of the hair bases can be shown to be approximately one-tenth of the total area of the adaxial side of the leaf. The other assumption which had to be made was that approximately a constant proportion of the hairs was broken. This is clearly not strictly correct, but, on the other hand, there is the consideration that the chance of particles being held up by the broken hairs is probably rather large. This is a difficult question to settle definitely, but one experiment can be carried out which throws some light on the problem. Four or 5 days after being rubbed a leaf develops the necrotic spots which constitute the symptom of aucuba mosaic. During the first 3 days the greater majority of the spots have been formed and the numbers do not appreciably increase thereafter. If the spots which have been formed are "cut out" in a disc of tissue as

above described it is found that the number of particles in each appears to be of the order of 20-30. If the tissue between the spots be taken, on the other hand, and after maceration in water be used as inoculum few spots if any develop on the treated leaves. Patches of ten discs were taken, and after maceration with the minimum of water were rubbed on the leaves. In only one leaf out of ten so treated did a single spot occur. It would appear from this experiment that the "unattached" particles on a treated leaf are relatively few, since there is evidence that the aucuba mosaic virus is comparatively resistant to drying and keeping.

If similar discs of tomato-leaf tissue be taken and macerated it was found that the number of particles present is very much larger than in similar discs of *N. glutinosa* tissue. The number of spots formed on *N. glutinosa* leaves after rubbing with a macerated disc of tomato-leaf tissue amounted to hundreds. It was in most cases impossible to count the spots which number over 600 per leaf. The multiplication of the virus in the tomato is, therefore, enormously greater than in *N. glutinosa*.

SUMMARY.

In this paper the symptoms of aucuba mosaic of tomato in *N. glutinosa* are described. A method is discussed whereby it is possible to count the spots formed after inoculation with juice diluted to different strengths. The fact that the number of spots formed is proportional to the amount of dilution is taken as indication of the particulate nature of the virus. A method is suggested for counting the number of virus particles present in a juice. It is shown that the amount of virus present in a juice does not increase after agitation or after treatment with proteolytic enzymes. With trypsin and diastase they were decreased. This decrease, it is suggested, is due to the adsorption rather than to the destruction of the virus. The amount of multiplication of the virus in the tissues of *N. glutinosa* is examined and compared with the much greater multiplication in tomato tissues.

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STREAK IN TOMATOES ASEPTICALLY GROWN

BY J. HENDERSON SMITH, M.B., CH.B.

(Mycological Department, Rothamsted Experimental Station, Harpenden.)

THERE has long been a theory that viruses may be filterable invisible stages in the life history of visible non-filterable bacteria. The idea is plausible in itself, and agrees well with the curious fact that in one or two cases specific bacteria are regularly found associated with particular virus diseases. The best instance is perhaps the well-known case of swine fever or hog cholera, a virus disease from which *Bacillus suispestifer* can be isolated so regularly that it was at one time believed to be the cause of the disease. But while facts of this sort are suggestive, there has been no direct evidence in favour of the theory. Recent work has made it increasingly probable that many bacteria can and do have filterable forms from which the original non-filterable organisms can again be obtained, but I know of no established case in which there has been obtained from a virus disease a bacterium capable, in either its filterable or non-filterable state, of reproducing the virus disease again. There has always been the possibility, even the likelihood, that the associated bacteria are secondary invaders which find in the virus-affected hosts a soil peculiarly adapted to their requirement.

The usual method of investigating this problem has been to begin with the virus-holding material, isolate from it, if possible, bacteria, and then endeavour to reproduce the disease with these organisms or obtain from them filterable material capable of reproducing it. There is in the literature of plant pathology one case recorded in which it was claimed that from a virus disease were regularly isolated bacteria capable of reproducing the disease. This is the streak or stripe disease of tomatoes, from which Paine and Bewley⁽¹⁾ isolated the organism *B. lathyri*, and believed that this bacillus on re-inoculation into tomatoes again produces the streak. But this has not been confirmed by other workers, and it is doubtful whether the authors would now be prepared to maintain their contention, at least as regards *B. lathyri*. It is, however, the fact that from tomato streak it is possible regularly to isolate specific bacteria, and it seemed worth while to approach the question from a different angle, inverting the usual method of investigation. It seemed possible to grow tomato plants aseptically from sterile seed, to inoculate these

aseptic plants with bacteriologically sterile virus-holding material and, when the disease was fully established, to ascertain whether the diseased plants contained the associated organisms. If they did, it would be good evidence that the bacteria had arisen from the virus. The following pages are a brief record of experiments carried out with this object.

Tomato streak is a disease characterised by extensive necrosis, which takes the form of black spots, often confluent, upon the leaves, and typically by black streaks upon the petioles and stems, where they may attain a length of 2 in. or more; it is usually accompanied by considerable mottling or patchy chlorosis on the leaves. It is a characteristic virus disease, transmissible by filtered juice and transferable to other Solanaceous hosts, notably to tobacco when it may be exceedingly destructive and is often accompanied by extensive and spreading necrosis of the pith. Bacteriological investigation of the necrotic tissues regularly yields bacteria of two main types, a white type and a yellow. There frequently also occurs a red yeast, but with this no detailed work was carried out, beyond ascertaining that it was not pathogenic on inoculation.

In view of the negative results obtained in the main object of the experiments only a short account of the white and yellow organisms will be given here. When first isolated, the white organism is a long, slender, markedly banded rod with ends that are often tapering. It grows freely on ordinary standard broth agar at 25° C., producing on plates large white dense slimy colonies, which tend to coalesce and grow over the whole surface; on slopes the slimy growth runs down the tube and accumulates at the bottom. This is the characteristic type regularly obtained on first isolation; but on culture there arises from it a second form, growing more slowly and producing smaller colonies with dry wrinkled surfaces and not tending to coalesce. This second form frequently appears at the margins of the mucoid growth, and may give it the appearance of having a dry feathered edge. It reverts quickly to the mucoid type, but by repeated selection can be so far stabilised that it remains true to type for several sub-cultures; it always, however, in our experience eventually produces again the mucoid type, which by its more rapid growth swamps the dry form in the cultures. In broth the mucoid type grows well, forms considerable deposit and a surface scum. At first the individual organisms are very long and banded, but after 8-14 days shorter forms appear, and the margins of the scum take on a dry wrinkled appearance. Occasionally on plates covered with mucoid growth, small papillated colonies develop, dark to transmitted light. All

these types are mycobacteria, with the usual characters of the group, converting phenol, growing on tap water and mineral salt agars, and producing the typical branched dendritic growth when the food supply is scanty or ill-balanced. In tomato juice which had been steamed, filtered, and sterilised by autoclave or repeated steaming, these organisms produce at the margin of the liquid small dark brown nodules, firmly adherent to the glass (cf. Bewley(2)). These have a crystalline structure and are of striking appearance. They are particularly well seen on tomato-juice agar, a colony being often surrounded by a ring of these nodules below the surface of the agar. They are not characteristic of these organisms, and can be produced in tomato extract by many bacteria (e.g. by many soil organisms) and some fungi (e.g. *Penicillium*). They would seem to be compounds of magnesium and calcium with fatty acids, and analogous to the bodies described by Laidlaw(3).

The yellow organisms, short bacilli, also form a small group of apparently closely related types. Although similar none was identical with any of the several strains of *B. lathyri* I was able to procure, and these so-called *B. lathyri* strains differed from one another in sugar reactions and other ways. They were obtained very regularly from the necrotic tissue, occasionally in pure culture but more commonly in association with the white organisms, and in the latter case they did not appear on the plates for 7-10 days, by which time the mycobacteria had usually covered most of the surface. On two occasions it has happened that from a piece of pith rubbed over an agar slope and left in the condensation water no growth developed for 35 days, but then the yellow bacteria grew out from the pith, and were readily sub-cultured. They are capricious in culture, and it is not unusual to have one plate covered with the small translucent smooth colonies and a duplicate plate without any growth at all.

Tomato seeds were sterilised by immersing them singly in 95 per cent. alcohol for 3 min., and then, after three washings in sterile distilled water, in a solution of mercuric chloride (1 gr. HgCl_2 , 500 c.c. water) for 12 min., during which they were repeatedly shaken. They were then washed in four changes of distilled water, and transferred with aseptic precautions to 2000 c.c. Erlenmeyer flasks stoppered with cotton-wool and covered with loosely fitting paper caps. Each flask contained a nutrient agar consisting of 700 c.c. of Brenchley's water-culture medium (KNO_3 , 1 gm.; KH_2PO_4 , 0.3; K_2HPO_4 , 0.27; MgSO_4 , 0.5; NaCl , 0.5; CaSO_4 , 0.5; Fe_2Cl_6 , 0.04; distilled water 1000 c.c.) and 7.5 c.c. agar, and had been sterilised in the autoclave the previous day. The seeds were

transferred singly, and inserted at the edge between the agar and the glass, care being taken that they lay at or only just below the level of the agar. Four or five seeds were put in each flask, which was then placed in the glasshouse. The treatment did not invariably sterilise all the seeds, and controls of 30-50 seeds in batches of five were always set up in Dunham's solution. The presence of infection on the seeds or contamination during the manipulations revealed itself by growth on the agar while the plants were developing; infected flasks were rejected, and were few at this stage.

Germination occurred after an interval which varied in different experiments from 5 to 16 days, a great delay compared with the 3-day period usual with untreated seeds, but was fairly complete, 80-90 per cent. of the seeds growing into plants. The plants grew well, though slowly, and after 5-7 weeks had three to four well-developed leaves and were ready for inoculation. This is difficult, and various methods were tried. The technique eventually adopted was as follows. Virus-holding juice from infected plants was filtered first through paper-pulp, then through an L_1 Pasteur-Chamberland candle, then through an L_3 candle, and was distributed in test-tubes in 5 c.c. volumes. The bacteriological sterility of the juice was tested by incubating some of these tubes, and by inoculation to broth. Scissor-shaped forceps with very long arms and broad blades had the blades covered with cotton-wool, and were sterilised in the dry oven. A pair of such forceps was inserted into a tube of virus juice until the wool was well moistened, then one or more leaves of each plant in a flask were crushed between the blades, one plant in each flask being left uninoculated. This method was quite satisfactory in effecting inoculation, but with the facilities available it was difficult to carry out aseptically, and the proportion of flasks infected was very high. The process of crushing the leaves required that each flask was open to the air for several minutes. Further, the flasks remained in the warm moist conditions of the glasshouse for 8-10 weeks or even more from the time the seeds were sown to the time the plants were removed for examination, and it was evident that in some cases contamination had occurred by passage through the cotton-wool plugs. The contaminations were usually fungal, but bacterial infection was not uncommon. The bacteria were usually cocci or spore-bearing rods of *subtilis* type; but on one occasion mycobacteria were obtained, and on two occasions yellow organisms resembling those found in streak infections. These were probably all aerial in origin; it is not unusual here to find similar yellow bacterial contaminations in work which has no connection with streak.

The plants under their unusual conditions developed good symptoms in 8-10 days, a slight increase in the incubation period above the normal, probably due to the slower growth of the plants. Mottling of the leaves was marked and occurred in almost every case. Necrosis was not always obtained, and typical streaking of the stems was rare, but streaking of the petioles and necrotic patches on the leaves was common. It sometimes happened that in the same flask one plant had well-marked necrosis, and another had mottle only without true streak; a small proportion of the plants died.

Two to three weeks after appearance of the symptoms the flasks were opened and the plants examined bacteriologically. The whole plant (sometimes two together) except the root system was minced and crushed in broth or Dunham's solution, and after 2-4 hours plates of standard agar and also of potato agar were prepared, pieces of crushed tissue being always included on the plates. The plates and broth (or Dunham) tubes were incubated at 25° C. Uninoculated plants were similarly treated.

The results of a single experiment may be given as an example. Twenty sets of plates were made from thirty-two inoculated tomatoes. Of these seven contained fungi, seven showed bacterial growth (which was neither yellow nor mycobacterial but was curiously fenestrated as if from the action of a bacteriophage); six were wholly sterile. The six sterile sets represented nine inoculated plants. Six sets of plates were made from six uninoculated plants; of these two gave fungal growth, two fenestrated bacterial growth, one both bacteria and fungi, and one only was sterile. At the same time five sets of plates were made from twelve inoculated tobaccos. Of these three gave fungal growth, one bacterial growth, one only was sterile. Three sets of plates were made from uninoculated tobaccos; of these two were sterile.

In all 137 tomatoes were inoculated, and examined, the number of tobaccos was much smaller. In the case of tomatoes 30 per cent. of the inoculated plants remained completely free from bacteria or fungi, although showing well-marked symptoms; from tobaccos only 20 per cent. sterile sets were obtained. These proportions are low for reasons given already. If one included in the figures the sets where fungi only without bacteria were present, the proportions would be much higher, but it is better to count them on the other side. They are enough to establish the facts that it is possible to obtain well-marked signs of streak disease without the appearance of the bacteria usually associated with it in the ordinary conditions of growth, and that when the plants are grown under aseptic

conditions throughout, these bacteria do not develop. Nothing has been found to indicate that these organisms are derived from the virus, or the virus from these organisms.

I have pleasure in acknowledging assistance given me by Miss H. van Straaten, of Wageningen, in some of the experiments made in 1931.

SUMMARY.

From the necrotic tissues of tomato plants suffering from streak, a virus disease, it is possible regularly to obtain bacteria which belong to one or both of two definite types. The same bacteria are to be found in tobaccos infected with the same disease.

If the plants are grown from sterile seed under aseptic conditions throughout and inoculated with bacteriologically sterile virus-holding streak juice, these bacteria do not appear although the plants develop well-marked signs of the disease.

No evidence has been found that the bacteria are derived from the virus or the virus from the bacteria.

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ZONE LINES IN PLANT TISSUES

I. THE BLACK LINES FORMED BY *XYLARIA POLYMORPHA* (PERS.) GREV. IN HARDWOODS

By ALEX. H. CAMPBELL, B.Sc.

(*From the Mycology Department, University of Edinburgh.*)

(With Plates XI-XIII.)

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I. INTRODUCTION.

THE zone lines formed by certain fungi in the invaded host tissues have presented a puzzling problem to mycologists. Their structure, formation and significance are little understood, while the accounts in the literature are generally obscure or speculative. Hubert (13) has pointed out the diagnostic value of zone lines, particularly those associated with incipient decay, but until we have more accurate knowledge about them, additional evidence is necessary in the identification of a rot. The confusion existing in the literature may be partly explained by the nature of the problem, since the zone lines may be formed by a number of different fungi in a variety of different ways.

White (30), referring to black lines in general, states that cultural or other evidence shows that when they do occur more than one species of fungus is at work. He says that black lines are well known to pathologists and are produced at the point of contact of two invading fungi in the

case of many pairs of wood-destroying fungi. Weir⁽²⁰⁾ has stated that "practically in every case where two distinct wood-destroying fungi occupy the same substratum, the mycelium or the wood decayed by each is sharply outlined or bounded by infiltrated zones of thylose-like deposits in the cells. These zones or layers are similar to the lines of demarcation often appearing in the rot of a single species and which separate the diseased from the healthy wood. The antagonism of the mycelium of one species to that of another is a marked characteristic of many wood-rotting fungi. The rot of *Fomes pinicola* and that of *Fomes fomentarius* in the wood of the same birch tree is always sharply separated by a conspicuous black line although the decay produced by each species is quite characteristic. The same evident antagonism is often true in the case of purely saprophytic species when occupying the same substratum." Hubert⁽¹³⁾ has made some observations on such zone lines and states that they occur between the rots of *Polyporus anceps* and *Lenzites sepiaria* in *Picea canadensis* and *Fomes applanatus* and *Stereum frustulosum* in *Quercus* sp. A double zone line may often be formed. No satisfactory account exists of the formation and microscopic structure of these zone lines formed between the mycelia of two different fungi on the same substratum, and the record of their occurrence depends chiefly upon the identification of the invading fungi from sporophores or the presence of typical rots.

Hartig⁽¹¹⁾ has described the formation of a black line in wood by the attack of a single fungus, *Armillaria mellea*. According to Hiley⁽¹²⁾ the honey fungus comes to occupy the cambium and from this position invades the wood on one side and the bark on the other. The extent of the invasion in the xylem is marked by a black line, one or more tracheides thick, and generally in the shape of a triangle with its base in the cambium. This black line consists of densely packed, bladder-shaped hyphae in the lumina of the tracheides and fibres. It has been stated by Hartig that the black line accompanies the advance of the rot and that the wood after its passage gives a cellulose reaction and speedily dissolves from the lumen outwards. Hiley has illustrated the method of movement of the line and has stated that the bladder hyphae are a more active stage of the fungus whereby the wood tissues are broken down much more readily. According to Butler⁽⁴⁾ the black lines of *Armillaria mellea* seem to be laid down in the position which they continue to occupy permanently. The whole process, he declared, is much more like the development of some defensive reaction on the part of the host or the parasite. Brooks⁽²⁾, referring to a disease of rubber caused by *Ustilina zonata*, stated that "these conspicuous black lines are caused by the fungus forming a kind of sclerotic plate in the

tissues, the difference between this and the typical sclerotium being that the latter does not include within it portions of the tissue of the host. These black zones differ from rhizomorphic strands in the same respect." Brooks, however, figures the black line as a black mass with no apparent structure, and his suggestion as to the nature of these black lines was not founded upon experimental evidence. *Nummularia pithodes* and a "*Xylaria* species" have been observed by the same author to cause similar black lines in wood. Sharples⁽²⁵⁾ describes the black line formed by *Ustulina zonata* in rubber as formed by the aggregation and massing of hyaline hyphae and later the deposition in the cells of a carbonaceous material. Panisset⁽¹⁶⁾ has described *Daldinia concentrica* as causing black zones in ash. She states that the significance of the black dichotomous hyphae, which are the principal constituents of the black zones, is unknown, but suggests that they may be compared with the brown, bladder hyphae of the *Armillaria* black line since both kinds lose their protoplasmic contents. Another suggestion is that the black zones represent an arrested development of ascocarps.

Rhoads⁽²¹⁾, in a paper entitled "The black zones formed by wood-destroying fungi," has dealt with black lines in a general way and was apparently under the impression that the structure of the black lines formed by the particular fungus he worked with was typical of the black lines formed by the other fungi. He dealt with the black lines caused by *Coriolus prolificans* (*Polystictus versicolor*) on *Hicoria minima*, and stated that the black zones in wood caused by fungi are in substance the same as those caused by wounds under natural conditions, being composed of natural decomposition products which infiltrate the cells and form droplets in the lumina. The continual occurrence of blackish zones between decayed and undecayed wood is due to the fact that the decomposition products are destroyed with the wood while new ones are formed from the wood as fast as it is attacked by the advancing fungus. The formation of the brown decomposition products depends upon (a) the presence of dead cells, (b) an optimum supply of moisture, and (c) sufficient oxygen to cause oxidation. He further stated that "the decomposition products whose formation is due to the action of wood-destroying fungi have proven to be a group of substances analogous to or nearly identical with the decomposition products, which arise under certain circumstances in dead wood that is entirely free from fungal attack and which have been known under the name of 'wound gum.' Their formation is greatly accelerated by the presence of wood-destroying fungi which greatly hasten the decomposition and hence the oxidation." There seems to be

no doubt that "wound gum" is capable of forming black lines, but it is by no means the only cause, as Rhoads has suggested.

Hubert⁽¹³⁾ has indicated that zone lines occur more commonly in pieces of infected wood most subject to desiccation. Hartig⁽¹¹⁾ states that the zone lines in oak wood infected with *Fomes igniarius* are due primarily to the entry of air into infected wood tissues. Lindroth⁽¹⁴⁾ figures a zone line formation just behind the freshly cut surfaces of birch wood infected with *Polyporus nigricans*. Hubert⁽¹³⁾ suggests that, "Desiccation and oxidation of the decomposition by-products seems responsible for the formation of these lines." Data on the appearance of zone lines in wood inoculated with pure cultures of wood-destroying fungi support this view. The zone lines invariably appeared during the periods when the moisture in the tubes and in the inoculated blocks had nearly evaporated. Zone lines were secured in the following cultures "on the hosts named; *Trametes pini* in *Picea sitchensis* heartwood, *Fomes igniarius* in *Populus tremuloides* sapwood, *Xylaria polymorpha* in *Tilia americana* heartwood, *Polyporus adustus* (?) in *Populus tremuloides* sapwood, *Hymenochaete rubiginosa* in *Populus tremuloides* heartwood, *Fomes applanatus* in *Populus tremuloides* sapwood, *Ganoderma curtisii* in *Populus tremuloides* sapwood and in pure cultures on malt agar, the mycelium extending to the cotton plug and forming a zone line across the plug." He continued that the data indicated that whenever freshly cut pieces of wood infected with certain fungi are placed in a dry room or left to dry in the open, characteristic zone lines are formed a short distance back from and parallel to the cut surfaces. Field observations often show zone lines in the upper portions of rotted stumps in the region where excessive drying occurred, roughly parallel to the cut surfaces from which evaporation took place. It seems probable that desiccation is the cause of a particular kind of zone line, but nothing is known of the microscopic structure of the lines, which Hubert following Rhoads believed to be produced by the oxidation of decomposition products similar in structure to "wound gum." It is not proposed to deal here with the extensive literature on "wound gum," but mention must be made of the more recent work of Brooks and Storey⁽³⁾ and Swarbrick⁽²⁶⁾ who maintain that the production of "wound gum" is the reaction of living tissues to invasion as against the decomposition origin postulated by Rhoads.

It seems, then, that in the present state of our knowledge, we must recognise the existence of zone lines in wood due to three distinct agencies. These are (1) the antagonism of the mycelia of two different fungi occupying the same substratum, e.g. between the rots produced by *Fomes*

pinicola and *F. fomentarius* in birch(29); (2) the action of the single mycelium of certain fungi, such as *Armillaria*, *Daldinia*, *Ustulina*, etc.; and (3) the production of "wound gum" stimulated by (a) natural wounding, (b) parasitic invasion, fungal or bacterial, or (c) desiccation(13). It will readily be seen that there are no easy criteria for judging the origin of zone lines macroscopically in wood. The author has collected and had presented to him a considerable number of specimens of wood rots containing zone lines which, from preliminary examination, appear to fit into the groups set out above. Before any comparative study of these lines for use in the diagnosis of decay can be attempted, it will be necessary to supply an adequate descriptive and anatomical account supported by cultural and experimental evidence of the typical members of each group. It is the object, then, of this paper to provide a description of the black lines formed by *Xylaria polymorpha* in hardwoods together with an account of their origin, formation and biological significance.

II. *XYLARIA POLYMORPHA* (Pers.) Grev.

Xylaria polymorpha is one of the commonest pyrenomycetes found on decaying stumps of deciduous trees. Ramshottom(20) lists this fungus as "common," and it is certainly of quite frequent occurrence on wood of *Fagus*, *Acer*, *Quercus*, *Fraxinus* and other hardwoods. According to Saccardo(23) *Xylaria polymorpha* has been collected by Mougeot on the decaying wood of *Pinus sylvestris*, while Therry has collected it on the stem of *Araucaria*.

The author had already in his possession several specimens of beech showing very irregular black lines believed to be caused by *Xylaria* and further obtained at the Royal Botanic Garden, Edinburgh, a stump of *Acer pseudoplatanus* bearing a luxuriant growth of *Xylaria polymorpha* fructifications (Plate XII, fig. 1). Sections cut from the stump showed, on examination, the presence of black lines in the wood. The fungal fructifications were identified as *X. polymorpha* (Pers.) Grev. Saccardo(24) describes five varieties of this fungus based chiefly on shape. In Lloyd's(15) opinion they are not worth recording because *X. polymorpha*, as its name implies, is exceedingly irregular in shape. The temperature variations and other climatic factors of the environment determine to a much greater extent the shape of the fungus than does the nature of the matrix. There are, however, the critical characters of the species with which it agrees. These are a solid, white stroma, black on the outside; a wrinkled surface due to contraction; perithecia that do not protrude and an ascospore

length from 24 to 32 μ . The exact ascospore size of this material was $25 \times 7 \mu$.

Single ascospore cultures were obtained from the fructifications on the *Acer* stump, while isolations were made from the black lines in the wood by cutting out in a sterile manner pieces of black line and transferring them to malt agar plates. The resulting pure cultures agreed in every respect. A number of isolations was made from the black lines in the beech wood and more ascospore cultures were obtained from batches of *X. polymorpha* from different localities. The cultures from these various sources were all closely compared and were found to be identical in their cultural characteristics.

III. CULTURAL CHARACTERISTICS.

The ascospores of *X. polymorpha* germinated readily by the brown outer membrane splitting down one side, as described by Vincens⁽²⁸⁾, to allow the emergence of the hyaline germ tube which gave rise to the mycelium. The colonies of *X. polymorpha* have appressed mycelium with indistinct zonation. The margins of the colonies are generally lobed, the lobes being separated by radial ridges which arise from the centre of the colony (Plate XII, fig. 2). On sterilised wood blocks the mycelium is at first very delicate with little zonation, the size and other features varying with the wood employed. Later, the whole block of wood becomes covered with a thick white mycelium, which eventually becomes quite black. Luxuriant vegetative growth with pronounced zonation is obtained when the fungus is grown upon sterile carrot slabs in the daylight (Plate XII, fig. 3). The zones apparently correspond to the alternate day and night periods, which form in turn bands of appressed and aerial hyphae. On artificial media the colonies are at first pure white in colour, but later, with the formation of dark hyphae below the white mycelial mat, they become neutral grey. When grown on oat agar the black incrustation of the dark hyphae appears after about a week, while at the end of 10 days erect stromata begin to form in the centre and at the margin of the colony (Plate XII, fig. 2). After a month's incubation at 25° C., when grown in tubes or a longer period in plates, the stromata reach 2 or 3 in. in height and about $\frac{3}{16}$ in. in diameter and are covered at the tips with a coating of light brown conidia.

The fungus has been cultured on a number of artificial media including the following: oat agar, Etter's⁽⁶⁾ medium, C.¹ medium, maize extract

¹ This medium is made up by mixing dry 35 gm. of beech sawdust with 100 gm. each of Quaker oats and maize meal. Fill the culture flasks to a depth of 2 in. and then saturate with a malt extract solution of 15 gm. malt extract in 1000 c.c. Sterilise.

agar, malt extract agar, potato dextrose agar, Czapek's solution agar, Dox agar, soil extract agar, starch agar, agar and gelatine. On the following sterilised plant parts, carrot, potato, and potato with the addition of 1 per cent. glycerine, excellent growth was obtained. The mycelium grew well on beech, *Acer*, ash, elm, oak, lime, and poplar blocks in large tubes ($8 \times 1\frac{1}{2}$ in.) sterilised by two exposures of 30 min. at 25 lb. steam pressure and on unsterilised beech blocks. Excellent mycelial growth and rapid stromatal production were obtained with oat agar, and Etter's medium. The growth on the modified Etter's medium was quite remarkable, stromata 6 in. high and some $\frac{1}{4}$ in. in diameter being produced. Malt extract agar, carrot, potato, potato with 1 per cent. glycerine and potato dextrose agar gave good growths while starch agar, Czapek's solution agar, Dox agar and soil extract agar gave much poorer results. According to Freeman (7) and Bronsart (1) *X. Hypoxylon* requires asparagin as a nitrogen source in the formation of stromata, while *X. polymorpha* requires ammonium nitrate. The addition of varying quantities from a trace to 1 per cent. of asparagin and ammonium nitrate to standard and synthetic media has failed to stimulate stromatal production in excess of the normal. On gelatine the fungus grew strongly showing an infundibulum liquefaction within a few days and finally complete liquefaction of the gelatine.

A number of isolations from wood while showing the characteristic *Xylaria* mycelium have remained sterile, producing no stromata. Guéguen (10) first noted this phenomenon and suggested that the development of *X. polymorpha* in culture was correlated with its seasonable development. He found that February to March was the best period for inoculation, and that cultures made before then tended to be sterile. Wolf and Cromwell (31) and Fromme and Thomas (9) have also observed that cultures may remain sterile for a considerable time before fructifying. According to the author's experiments the time of inoculation does not seem to have any effect in the production of stromata.

It was found that the most convenient temperature for growing cultures was 25° C. with the optimum about 27° C. Wolf and Cromwell (31) investigating a *Xylaria* root rot of apple found that the most luxuriant growth occurred in cultures kept in an ice-box at 11–13° C. In the case of *X. polymorpha* mycelial growth falls off below 16° C. and is very poor at 10–12° C.

When cultures are sealed up with a pyrogallic acid and caustic soda mixture to produce anaerobiosis, the growth continues slowly for a few days and then stops. The mycelium is eventually killed. If the cultures

are grown in sealed bottles with confined air, the mycelium remains white and in place of the stromata white sterile humps are formed. These humps are seldom more than $\frac{1}{2}$ in. high.

IV. DEVELOPMENT OF THE FUNGUS IN CULTURE.

The young filaments produced by the germinating ascospore are hyaline, branched and densely granular, with an average diameter of $1-2\mu$. Later, the hyphae lose their granular contents and become more closely septate and brown in colour. The transition from hyaline to brown hyphae in culture is a gradual process with a number of easily recognisable intermediary stages. The first stage is seen in the enlargement of the hyaline hyphae from a diameter of about $1.5-3\mu$ and the appearance of a greater number of vacuoles in the protoplasm. Later, the walls thicken and closely placed septa make their appearance (Plate XIII, fig. 4). The hyphae are now a uniform brown colour due to the deposition on the walls of a brown substance. About this stage the granular protoplasm finally disappears and its place is taken by clear contents. The long, straight hyphae with the well-defined septa begin to darken in colour, while the single cell units swell up and become bladder-shaped with a diameter from 4 to 10μ . These bladder cells which are now dark brown in colour tend to aggregate into black masses in which it is exceedingly difficult to differentiate the unit cells. This is practically the condition that is found on the black line in wood.

Eventually stromata appear in the cultures (Plate XII, figs. 2 and 5). When grown on *C.* medium these may attain a height of 6 in. with a $\frac{1}{4}$ in. diameter and are generally quite cylindrical and unbranched. The stromata present an odd appearance, having large globules of exuded water adhering to the surface, and differ considerably in appearance from those of *X. polymorpha* in nature. Cultures grown upon oat agar, with the addition of beech-wood sawdust, in large dishes have shown after 3 months' incubation at 25°C . stromata about $\frac{3}{4}$ in. high and exactly like miniature *X. polymorpha* fructifications as they appear in nature. In addition sterile coremia as have been described by Guéguen⁽¹⁰⁾ were prominent on this medium. When cultures were grown on oat agar in Erlenmeyer flasks at 25°C . until the stromata were well developed and then transferred to a cooler incubator at 17°C . the stromata became covered with brown hairs about 3 mm. long which projected at right angles to the stroma and extended from the base as far as the conidial bearing region at the tip. A single hair generally consists of a number of long, straight, unbranched hyphae, which are distinctly septate and

brown in colour, arranged parallel to each other and closely adhering to form a single unit. The presence of these hairs gives the stroma a very curious appearance (Plate XII, fig. 7). Another feature which results from the exposure to slightly lower temperature is the branching of the stroma. Generally when grown throughout at 25° C. the stromata remain straight and unbranched; if, however, the culture is exposed to a temperature of 17° C. for 24 hours the growing point at the tip appears to be checked and there is no further elongation of the stroma. Very soon, however, a whorl of new shoots, from two to four in number, makes its appearance from just below the checked tip and in this way the growth of the stroma is continued. This process of checking the tips can be carried out a number of times to produce stromata bearing successive whorls of branches. It might be suggested, since the shape and appearance of the stroma in culture can be varied so much, that the great irregularity in shape in the genus *Xylaria* and in particular in the species *polymorpha* may be due to the occurrence of this phenomenon in nature.

The conidia, which appear as a brown coating at the tip of the stroma, are hyaline and elongated oval in outline with a blunt truncate pedicel. They measure $10 \times 3.5 \mu$ and are borne on palisade-like conidiophores. The conidia germinate readily to produce a single germ tube which gives rise to the mycelium, and in this way seven successive generations of *X. polymorpha* have been raised in culture.

A number of media have been experimented with, but so far all attempts to obtain perithecia and ascospores in culture have been unsuccessful. Complete stromata have been sectioned, but no sign of perithecial formation has been detected. With the exception of Freeman(7), who states that ascospores were produced in cultures of *X. Hypoxylon* on steamed blocks of elm wood in 6 or 8 weeks, ascospores are unrecorded in culture for *X. Hypoxylon* and *X. polymorpha*.

V. BLACK LINES PRODUCED IN INOCULATED WOOD.

A number of wood-block cultures were set up to test the production by *Xylaria* of stromata and black lines on a wood substratum. The woods used were beech, *Acer*, lime, elm, ash, oak and poplar and at first young branches about $\frac{3}{4}$ in. in diameter and cut into 2-in. lengths were used as the test-pieces. Later, it was found that the hyphae penetrated the wood much more readily if blocks were used which had been cut as $\frac{1}{2}$ in. thick transverse slices of the trunk and then divided into pieces 3×1 in., as in these pieces the largest possible area of "end-wood" was available for the entry of the fungus. The blocks were soaked in water for 24 hours

before placing them on a cotton-wool base in large tubes ($8 \times 1\frac{1}{2}$ in.), and varying quantities of pure water were added before the whole was sterilised at 25 lb. steam pressure for 30 min. The test-pieces were inoculated with conidia and mycelium from *X. polymorpha* cultures and from isolations from black lines in beech and *Acer*. The cultures were incubated at 25° C. for 12 months and then the blocks were examined for black-line production.

The test-blocks were covered with a dense, felty mycelium, brown-black in colour and forming a thick crust on the wood. About 80 per cent. of the cultures bore stromata and conidia, as is shown in Plate XII, fig. 7. When the mycelium was scraped off the blocks black lines and ovals were found which were identical in appearance with those in the *Acer* stump but not nearly so numerous. In most of the cultures the black lines were superficial and did not penetrate the wood more than a short distance. It was observed that the cultures with little surface mycelium generally produced quite a large number of black lines in the wood. The cultures which had an exceedingly thick crust of mycelium, due to a larger amount of water being present in the tubes when inoculated, yielded only a delicate black tracery at the surface of the wood, although the thinner hyaline hyphae were present in the wood in large numbers. It is suggested that insufficient aeration may be a limiting factor in the formation of the black lines, as it has been shown previously that cultures grown in confined air do not develop the black hyphae which compose the black line but only the thinner hyaline hyphae and remain quite white in colour. A very striking feature, however, was the presence of distinct black lines in the cotton-wool at the bottom of the tubes (Plate XII, fig. 6). These black lines could be dissected out with care and were found to consist of a shell, complete, except at the point where the black lines came into contact with the glass, which thus prevented their full development; so that the black circle seen in the cotton-wool is really a section through an egg-shaped body which has the black line for its circumference. The significance of these ovoid bodies will be dealt with in a later section.

Since cultures raised from single ascospores of the *X. polymorpha* fructifications on the *Acer* stump and from the isolations from black lines in the stump have been found to be identical in cultural characteristics and stromatal and conidial formation, there could be no doubt as to the identity of the fungus causing the black lines in the *Acer* stump. Isolations from black lines in beech, in which wood they are most commonly found, have yielded cultures identical with those of *X. polymorpha*, and the morphology of these black lines agrees in every particular with that of

the *Acer* wood. In addition, conidia and mycelium of *X. polymorpha* have produced black lines in test-pieces of beech, *Acer*, lime, elm, ash, oak and poplar, as have also isolations from black lines in beech and *Acer*. It seems, however, that *X. polymorpha* is capable of producing black lines in a number of hardwoods just as its allied genera, *Ustulina* (2) and *Daldinia* (16), have already been shown to produce black lines in rubber and ash respectively.

VI. MORPHOLOGY OF THE BLACK LINE.

Hubert (13) has figured black lines in the trunk of *Acer rubrum* produced by *X. polymorpha*. According to him the zone lines are formed round areas which originate from the various foci of infection and produce double lines where such areas meet. Petch (17, 18, 19), investigating a root disease of rubber and tea caused by *X. Thwaitesii* Cooke, has observed that black lines and ovals may be present and that the hardness of the wood, despite its intense discoloration, was a striking feature. Fromme (8) stated that black lines or blocking layers are sometimes seen in the wood in the case of apple root rot caused by *Xylaria* spp. Brooks (2), investigating a disease of rubber caused by *Ustulina zonata*, described conspicuous black lines about $\frac{1}{32}$ in. thick occurring in the wood. The same author has found *Nummularia pithodes* (B. and Br.) Petch and a species of *Xylaria* (probably *X. Thwaitesii*) causing similar black lines in the wood of rubber.

The author had the opportunity recently of examining a complete 60 ft. specimen of *Fagus sylvatica* which had been apparently killed by *Fomes applanatus*. At the base of the trunk, some 2 ft. 6 in. in diameter, the black lines of *X. polymorpha* were extremely numerous although no fructifications were present. The black lines extend in what appear to be vertical plates which may fuse frequently in the ascent of the trunk. These plates when seen in section appear as thick lines, hair lines, double lines, ovals or "islands," all branching and then anastomosing to form crazy patterns in the wood. Plate XI, fig. 1, shows part of an oblique-transverse section 4 ft. from the base and shows the black lines distinctly. Apparently no attempt is made by the fungus to follow the easiest path through the wood, as the black lines pass through all types of tissue and penetrate both bark and sapwood as can be seen in this photograph. A remarkable feature is the soundness of the wood, which is extremely hard, especially at the black line, of normal colour and free from brashness. Plate XI, fig. 2, shows a transverse section of one of the twin trunks cut 7 ft. above the ground. "Islands" and ovals are especially prominent in

this section, which may be explained by the fact that the black lines, as they appear in section, are actually the circumferences of a number of ovoid or narrow ellipsoid bodies whose long axes are parallel to the tree axis. Thus these "islands" represent sections cut through these ovoid bodies close to the apices. The actual number of black lines present in this top section is much smaller than at the base, so that it can be concluded that the furthestmost extent of the black lines has almost been reached. Further sections confirm this, as the black plates converge to their apices and finally disappear 9 ft. from the base.

When the invaded wood tissues are sectioned and stained with safranin and picro-aniline blue(5) a number of minute hyphae stain up bright blue around the black line. The black line itself consists of a dense mass of brown bladder cells occupying the lumina of the vessels, fibres and cells of the medullary ray in the path of the line. The black line is best examined in unstained longitudinal radial sections (Plate XIII, fig. 3) which must be as thin as possible so that in microtoming longitudinal sections are preferred to transverse ones. Examination of many of the black lines produced in culture blocks has shown a number of stages in the formation of the black line. It appears that first of all the position which the black line is to occupy becomes marked out by the aggregation of thin, hyaline hyphae. Later, the hyaline hyphae swell up and become closely septate forming the brown bladder cells as in culture. The bladder hyphae first make their appearance in rows of isolated patches of irregular shape, then gradually the intervening spaces become filled with the brown hyphae and the line begins to assume a distinct and clear-cut appearance with the close packing of the hyphae in every available cavity within the limits of the black line. Some of the bladder cells then collapse and their contents stain the walls of the vessels, penetrate the pits between the cells of the medullary ray and fill the interstices between the other bladder cells. The result is to produce in a complete section the effect of a closely packed barrier through which penetration must be nearly impossible. In addition, this packing of the swollen hyphae lends mechanical support and rigidity to the black line which is always very much harder than the surrounding wood. Indeed, so tightly are these swollen hyphae packed that it has been found possible in sufficiently decayed wood to dissect out the black lines in the form of sheets several square inches in area. These black plates consist only of the swollen hyphae and remnants of the walls of the cells in which the hyphae were formed.

The brown substance which stains the cell walls and fills the pits is of unknown composition, and very little is known about its formation. It

was found to be insoluble in acetone, alcohol, benzol, carbon disulphide, chloroform, methyl ether and hot water. Concentrated sulphuric, hydrochloric and nitric acids and strong potassium, ammonium and sodium hydroxides, hot or cold caused no reaction. Iodine, however, gave a blue reaction along the black line when microsections were tested, and Eau de Javelle completely bleached the line after 24 hours.

Hiley has suggested that the bladder cells in a somewhat similar black line caused by *Armillaria mellea* are "a more active stage in the metabolism of the fungus" which breaks down the wood tissues more readily. It must be remarked, however, that if such is the function of the hyphae of the black line, they seem singularly ill-adapted to their function. It is to be expected that hyphae of high functional activity would present the maximum surface area for assimilation and also be well spaced to allow of sufficient aeration. This does not seem to be the case with the hyphae of the black line. In fact, the line hardly varies in breadth but remains clear-cut with no apparent tendency to spread. This point will be dealt with more fully in a subsequent communication on the black lines caused by *Armillaria mellea*, but meanwhile it will be sufficient to add that in the sounder wood as shown in Plate XI, fig. 1, tests reveal scarcely any delignification and certainly no perceptible difference in the delignification on either side of the black line. On the other hand wood in a more decayed condition as in Plate XI, fig. 2, shows what at first sight appears to be a different condition of affairs. Towards the left-hand corner of this specimen and in the centre can be seen areas which judged by the difference in their colour seem to be at different stages of decay. These areas are bounded by black lines, so that it might be thought, and not without some reason, that the black lines represented the lines of advance of the fungus from different foci of decay. In the centre of the specimen is a considerably rotted area which has been caused by the *Fomes*. On top of this heart rot the black lines are superimposed, making the whole appear, with its differences in light and shade, not unlike a patchwork cover. If it were postulated that these black lines occur in the position they do quite fortuitously, the apparent differences in decay on either side of the black line could be explained by reference to an anatomical feature of the black line. This feature is the closely packed bladder hyphae, by virtue of which the black lines form complete barriers, such as might quite easily prevent the further spread of fungi and bacteria. To reconstruct what happened inside this tree it is necessary to get a mental picture of the successive invaders. The initial *Fomes applanatus* rot gaining access at the base spread upwards killing the wood and rotting the heart area. The brown

zone which marks the advance of the *F. applanatus* in living wood cannot be easily seen, as this specimen had been standing dead for some time and later saprophytic fungi have practically removed the brown zone. As one of the first of these saprophytic fungi came *Xylaria*, whose hyaline mycelium spread throughout the dead tissues without causing any marked change in their appearance. Eventually the *Xylaria* developed its black lines so that all subsequent invaders entering by the same route as the *Xylaria* tended to be isolated by the barriers presented by the black plates, and thus arose the areas with different degrees of decay separated by black lines. To confirm this hypothesis a number of cultures was made from the various rot areas and several unidentified fungi along with *Fomes applanatus* were isolated from the typical rot, while *Xylaria* was only obtained after careful isolation from the black lines. In many cases bacteria seemed to be by far the chief invaders. Bacterial counts by direct observation and by dilution of equal weights of rotted tissue and subsequent platings have established without a doubt that there may be a considerable difference in the number of bacteria in adjoining areas separated by a black line. So that the impermeable nature of the *Xylaria* black line is responsible for the differences in decay in adjoining areas by the limitation it imposes on the secondary fungi and bacteria, which so commonly invade the later rots of *Fomes applanatus*.

VII. ZONE LINES OF SUPERIMPOSED ROT.

Not a little of the confusion existing over the formation of zone lines by wood-rotting fungi becomes understandable by reference to the specimen shown in Plate XIII, fig. 5. This photograph shows part of a beech-block attacked by a typical heart rot of *Fomes applanatus*. The region marked 1 is apparently normal wood, in colour and texture, and as yet uninfected. On the right of it and marked 2 is a dark brown band similar in appearance to that which has been described by White (30) as marking the advance of a *F. applanatus* rot. Section 4 consists of unnaturally white, much decayed wood, which represents the typical rot stage of the *Fomes* attack. Throughout this area and closely appressed to the brown band occur thin black lines (3) which have been identified culturally and morphologically as *X. polymorpha*.

The microscopic examination discloses that section 1 of the wood is normal with no hyphae or tyloses. Section 2, as can be seen in Plate XIII, fig. 6, contains a few hyphae, but a more prominent feature is the presence of tyloses which completely plug the vessels, while the parenchymatous cells and tracheides alongside are filled with a dark brown substance

known as "wound gum" (*w.g.*). The "wound gum" is also found filling up the spaces between the tyloses in the vessels and in a number of cases in the tyloses themselves. It is the "wound gum" which is responsible for the dark colour of this portion of the wood. The black line marked 3 is a normal *Xylaria* black line with the difference that in this case the line also contains tyloses which were formed first as a result of the *F. applanatus* attack and in consequence the bladder mycelium has been compelled to fill up the only remaining space in the vessels, which are more or less isolated pockets between the tyloses and in the lumina of the tracheides and parenchymatous cells. In spite of this, however, the line loses none of its distinctness, while every available space within the limit of the line is packed with hyphae and stained with the brown pigment which always accompanies the *Xylaria* bladder hyphae. This pigment is quite distinct and must not be confused with the "wound gum." Both, in this case, occur within the black line; the "wound gum" occupying the lumina of the tyloses is characterised by its granular and fractured appearance in mounted sections while the *Xylaria* brown pigment is always transparent and does not seem to fracture. In section 4 the wood is considerably delignified, and the hyphae of later saprophytic fungi and bacteria are present in large numbers. The "wound gum" has disappeared, having been apparently bleached or absorbed by the hyphae, and while the tyloses remain for some little distance from the black line they, too, are eventually broken down and absorbed by the invading fungi and bacteria.

The various sections of the wood are sharply delimited and of extraordinarily different appearance. The specific gravities of the sections 1, 2 and 4 were determined by a volumeter method of water displacement and were found to be of the following approximate values:

s.g. sound wood, section 1	0.72
s.g. infiltrated wood, section 2	0.89
s.g. decayed wood, section 4	0.48

It will be seen from these figures that the brown zone is considerably heavier than normal wood, and that the decayed wood is very much lighter than normal.

White⁽³⁰⁾, in his study of *F. applanatus*, refers to the brownish discoloured zone as marking the extreme limit of the advance of the fungus and making the line of demarcation from the sound and yet uninfected wood very distinct. The brown coloration is due to the "wound gum" which is found in the lumina of the parenchymatous cells, in the vessels and impregnating the walls of the tyloses. As neither the "wound gum"

nor the tyloses occur in the sound tissues beyond the brown band, they must be regarded as a pathological condition and a phenomenon which is never found in sound wood. There is an extensive literature on the subject of "wound gum" but, briefly, there are two divergent theories at present in existence. One opinion maintains that "wound gum" originates after the death of the cells as an oxidation product, while the other declares it to be a vital reaction and a secretion from living protoplasm. The tyloses, however, cannot enter this discussion as they are only produced by living cells. But at the moment the evidence is in favour of both "wound gum" and tyloses being formed as a vital reaction of the host to injury, mechanical or fungal, since the artificial production of "gumming" by the injection of toxins (3) and the considerable increase in the specific gravity of the infiltrated wood are unanswerable arguments against the decomposition origin of "wound gum." White summed up the position by stating that the brown band with its "wound gum" and tyloses may be regarded as the best criterion we have at present as to the parasitic nature of an invading fungus.

Portions of the infiltrated wood of section 2 have been cut out, soaked in water, sterilised and inoculated with *X. polymorpha* mycelium. Growth on this substratum proved to be very scanty, and after 12 months less than 1 mm. depth of the "wound gum" had been digested, which shows that the "wound gum" tends to limit the spread of the fungus in the wood. The *Fomes applanatus* mycelium entering as a heart rot would gradually work towards the periphery, its advancing hyphae stimulating the production of tyloses and "wound gum" from the living wood, while the hyphae behind gradually assimilate and destroy these products of invasion. There is reason to believe that the *Fomes* rot proceeds slowly over a number of years, being delayed probably by the host's reaction. As all the tissue behind the brown zone consists of dead cells, the *Xylaria* on entering would ramify quickly through this tissue and eventually the black plates would be formed. It seems reasonable that the ramifying *Xylaria* mycelium would tend to be checked on reaching the brown zone, and in consequence of the aggregation of hyphae the black line would arise in the position which it is seen to occupy close to the brown zone. It is probable that the *Fomes* mycelium died out before the entry of the *Xylaria*, but in any case no further advance was made by the *Fomes* after the formation of the *Xylaria* black line. Thus it will be readily appreciated how difficult the identification of a rot from zone lines becomes when, as in this case, the zone lines of two different fungi, a parasite and a saprophyte, are found superimposed on each other.

VIII. FORMATION OF THE BLACK LINE.

Infection of a wood block by conidia or ascospores of *Xylaria polymorpha* results in a thin hyaline mycelium which ramifies through the wood. Eventually the hyaline mycelium tends to aggregate, thereby marking out the zone where the black line will appear. From the examination of the black lines in inoculated wood blocks the development of the line can be followed from the early stages, when the brown mycelium occurs in more or less scattered patches, through the period of gradual extension of the bladder hyphae until the complete circumference of an ovoid-shaped body is formed. This ovoid body consists of the black peripheral zone of bladder hyphae and inside a matrix of practically unaltered wood tissues with remarkably few hyphae present. It is important in view of the various functions which have been attributed to black lines, to emphasise that the bladder hyphae do not move from the position in which they are formed. This fact has been established by the observation and measurement of the black circles formed in cotton-wool at the bottom of the culture tubes. In Plate XII, fig. 6, black circles can be seen in the cotton-wool which is practically pure cellulose. These circles represent sections through ovoid bodies, which have been produced by the black plates forming a shell around a matrix of cotton-wool, so that a complete circumference of bladder hyphae has been formed except where further development has been prevented by contact with the glass. In the wood a large number of these bodies is formed, and as their development is limited by the available space they become closely packed and irregular in shape, producing the characteristic double lines where the circumferences of two adjacent bodies approach or a single line where they become contiguous over a part of their area. When a portion of one of these bodies becomes exposed at the surface it gives rise to an effused black mycelium from which develops the stroma or fructification of the fungus and upon which are borne the conidia and the perithecia.

IX. SIGNIFICANCE OF THE BLACK LINE.

The families Diatrypaceae, Diaporthaceae, and Xylariaceae of the Sphaeriales are distinguished by the immersion of their perithecia in a stroma. These three families have been separated, as the Diatrypaceae and the Diaporthaceae are simple stromatic forms with the stromata only slightly raised from the substratum while the Xylariaceae has developed fully individualised fructifications independent of the substratum. It is hoped by a consideration of the black lines produced by the Xylariaceae

to suggest that the stromatic relationship of these three families is of a closer degree than was formerly thought to be the case.

The simplest form of stroma is seen in *Eutypa* of the Diatrypaceae where the perithecia are scattered singly but united by a black crust on the surface of the substratum. Ruhland (22) has given the name protostroma to this primitive type of stroma. The advance from the protostroma type is by the invasion of the hyphae and the development of the stroma in the substratum which result in the formation of an entostroma bounded by a black zone composed of swollen hyphae. According to Ruhland (22) *Diatrype disciformis* is one of the simplest of the type which is characterised by the differentiation of the stroma into an ectostroma and entostroma. In this fungus the ectostroma is formed as a plectenchymous disc between the bark parenchyma and the periderm where it raises the periderm into the form of a flat pustule. Eventually with the enlargement of the ectostroma the periderm is ruptured and the ectostroma protrudes and forms conidia around the base. Meanwhile hyphae from the plectenchymous disc which formed the ectostroma have penetrated the bark parenchyma to give rise to the entostroma, which is composed of fungal hyphae and bark parenchyma and is bounded by a black zone. Perithecia are developed in the entostroma, the upper layer of which forms a black sclerotic plate surrounding the necks of the perithecia. The formation of the hard sclerotic plate completes the separation of the entostroma and the ectostroma and shortly afterwards the latter is cast off leaving the entostromatic plate exposed. Ruhland in his study of the stroma-forming Sphaeriales has chosen this formation of ectostroma and entostroma as seen in *Diatrype* as one of the simplest conditions of the diplostromatic group. He suggests that from this stage the general tendency has been the retention of the ectostroma, its reduction and eventually its fusion with the entostroma.

The simpler genera of the Xylariaceae, as, for example, *Nummularia*, show a very close relationship to the Diatrypaceae and Diaporthaceae in the production of a definite entostroma bounded by a black line in the substratum. The entostroma is mainly composed of the parenchymatous tissue with few hyphae, while the upper effused portion that forms the black fructification consists only of fungal hyphae. The perithecia are imbedded in the bark cells and their necks extend through the plectenchyma to the surface. The author has found black lines in the substratum of *Nummularia discreta*, *Ustulina vulgaris*, *Hypoxylon coccineum*, *Daldinia concentrica*, *Xylaria polymorpha*, *X. carpophila*, and *X. filiformis*. These black lines on microscopic examination were found to be composed of

tightly packed bladder hyphae, which vary in size in the different genera, and dark-staining pigment filling up the pits and the lumina of adjoining cells. The morphological structure of the black lines in the Xylariaceae, and also, for example, in *Diaporthe*, is so remarkably similar that it is difficult to deny their homology. And since *Nummularia* is regarded as having an entostroma bounded by a black line, it is reasonable to infer that the black lines in the other genera also represent the marginal zones of entostromata in the substrata. A sequence in the development of the "stroma" or fructification can be followed from the *Nummularia* type, where there is a considerable entostroma in relation to the bulk of superficial "stroma," through *Ustulina*, *Hypoxylon* and *Daldinia*, in which the "stroma" assumes a definite form, to the erect, shrub-like fructification of *Xylaria*. It might be expected that there would be a reduction in the entostroma in proportion with the increase in the "stroma." This may be the case in some genera, as for example *Hypoxylon*, but certainly in *Xylaria* the amount of wood bounded by the black lines is immensely greater in volume than the fructifications.

An examination of the genus *Xylaria* throws more light upon the actual morphology of the entostroma. The Tulasnes (27) have depicted a number of species of *Xylaria* with black lines in the substratum, and although they describe *Xylaria* as perennating by concealed mycelium in the wood and mention that the substratum is "marbled by the innate mycelium," they apparently did not correlate the black lines in the case of *Xylaria* with the black marginal entostromatic zone in *Nummularia* and *Diaporthe*. In the case of *X. oxyacanthae* growing on the fallen fruits of *Crataegus Oxyacantha*, mention is made of the fruits being "marbled with black veins within their substance," but no suggestion is made as to their significance.

The Tulasnes described and figured *X. pedunculata* growing on rabbit dung as having a stroma, subterranean, sclerotoid, ovate, globose or very irregular, giving rise to a slender strand which swells up to form a head when it emerges above the dung. The perithecia are usually borne in this head, but the strand or the submerged stroma should they become exposed may bear perithecia as well. It seems, therefore, that there is no inherent difference in the potentialities of the stroma, strand or normally fertile head, but that their position determines their fertility. A terrestrial species, *X. bulbosa*, is also described as having a "tuber of the size of a small nut." It is logical to classify these "stromata," since they are completely immersed in the substratum, as entostromata, and to deduce, since they can bear perithecia, that they have the same constitution as

the normal fructification. The fact that *X. pedunculata* and *X. bulbosa* grow on a substratum which can be readily broken up has led to the recognition of the "tuber" or "sclerotoid stroma" which gives rise to the normal fructification. In *X. polymorpha* and *X. carpophila*, which grow on woody substrata, the hardness of the matrix has delayed the recognition of the fact that their fructifications, too, arise from a "tuber" and are not, as has been stated, independent of the matrix. No more striking example of the formation of this "tuber" could be had than the black ovoid bodies which were produced in the cotton-wool. The Tulasnes applied the term "stroma" to the subterranean "tuber" in *X. pedunculata* and seemed to recognise the homology of the swollen head, strand and stroma of the fungus, since they report that all three parts may produce perithecia, but at the same time they apparently believed that the whole structure of *X. pedunculata* was equivalent to the fructification alone of *X. polymorpha*.

Since the presence of black lines in the substratum of the Diatrypaceae and Diaporthaceae and their connection with the actual fructification as an entostroma have long been recognised, it seems remarkable that the black lines in the substratum of the Xylariaceae although observed should not also have been connected with the fructification as an entostroma. The author has observed that the black lines in the Diatrypaceae and Diaporthaceae are composed of swollen hyphae comparable to those occurring in the Xylariaceae, so that it seems reasonable to adduce their homology and describe the *Xylaria* black lines as marginal zones delimiting the entostroma in the substratum. The term stroma has by authority of long usage been applied to that part known as the fructification, while, morphologically, the stroma must include both the external fructification and the part within the substratum. This part within the substratum must now be recognised as the entostroma and the black plates which delimit it as the entostromatic zones. It must be emphasised, however, that the fructification and the entostroma are in no way separate structures, but that the fructification in *Xylaria* is simply an extension of the entostroma, which, having reached the surface, assumes a form adapted for the function of bearing conidia and perithecia.

X. SUMMARY.

A brief account is given of the literature on zone lines in wood, and a preliminary attempt is made to classify the zone lines into groups in order to provide a basis for systematic research and to facilitate the statement of results. A single fungus, *X. polymorpha*, suspected of causing black lines in hardwoods, is dealt with morphologically and culturally.

The development and stromatal production of the fungus on artificial media and wood blocks are described. *X. polymorpha* is established as a black line producing fungus by its production of black lines in inoculated wood blocks. The morphology of the black lines is described in detail and their actual formation has been observed in cotton-wool. As a result the suggestion is made that the black lines are the marginal zones of entostromata in the substratum comparable to those occurring in the *Diaporthe*. The black lines produced by the genera *Nummularia*, *Ustulina*, *Hypoxylon* and *Daldinia* in the substratum are stated to be of similar structure and significance.

An account is given of a *X. polymorpha* black line superimposed upon the zone line formed by the attack of *Fomes applanatus* on beech wood. The confusion existing in the literature on the zone lines of *F. applanatus* is partly attributable to this not uncommon phenomenon. The value of zone lines as criteria in the diagnosis of wood rots is discussed and some of the difficulties in the identification of a zone line in a typical rot are pointed out.

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EXPLANATION OF PLATES XI—XIII.

PLATE XI.

- Fig. 1. Part of a transverse-oblique section of a beech trunk. The irregular black lines of *X. polymorpha* show up clearly. $\times \frac{1}{2}$.
- Fig. 2. Transverse section of the same beech trunk seven feet from the base. Black lines of *X. polymorpha* can be seen in the heart rot delimiting areas of widely different appearance. Double lines and ovals are particularly numerous. See description in text, pp. 135–6. $\times \frac{1}{2}$.

PLATE XII.

X. polymorpha (Pers.) Grev.

- Fig. 1. Fructifications in nature on stump of *Acer pseudoplatanus* at the Royal Botanic Garden, Edinburgh. $\times \frac{1}{2}$.
- Fig. 2. 14-day culture on oat agar with young stroma arising at the centre. Note the lobed margin and radial ridges. $\times \frac{1}{2}$.

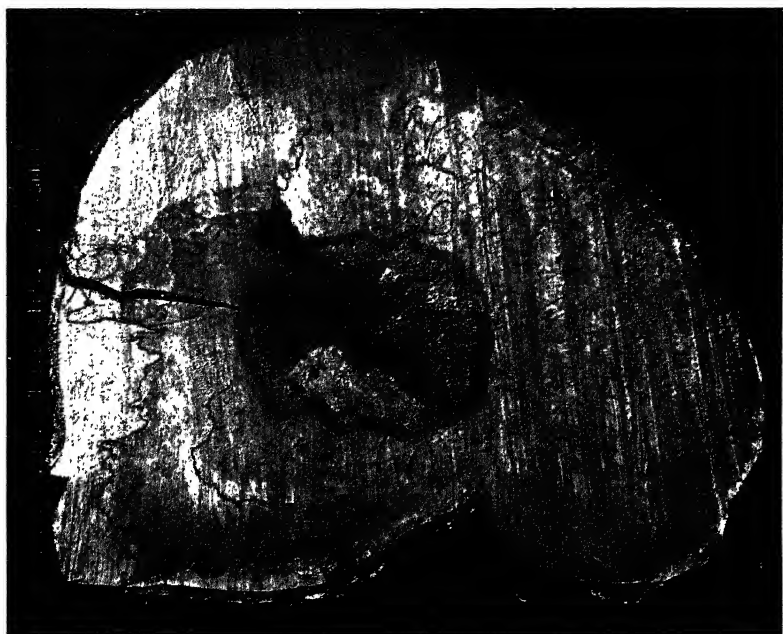
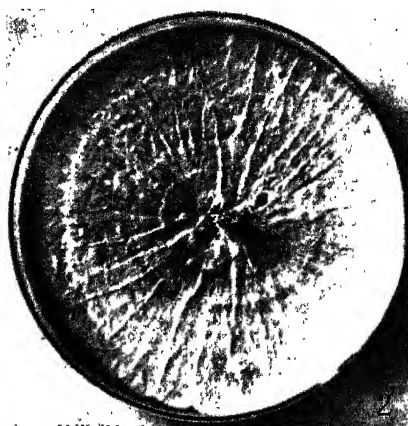


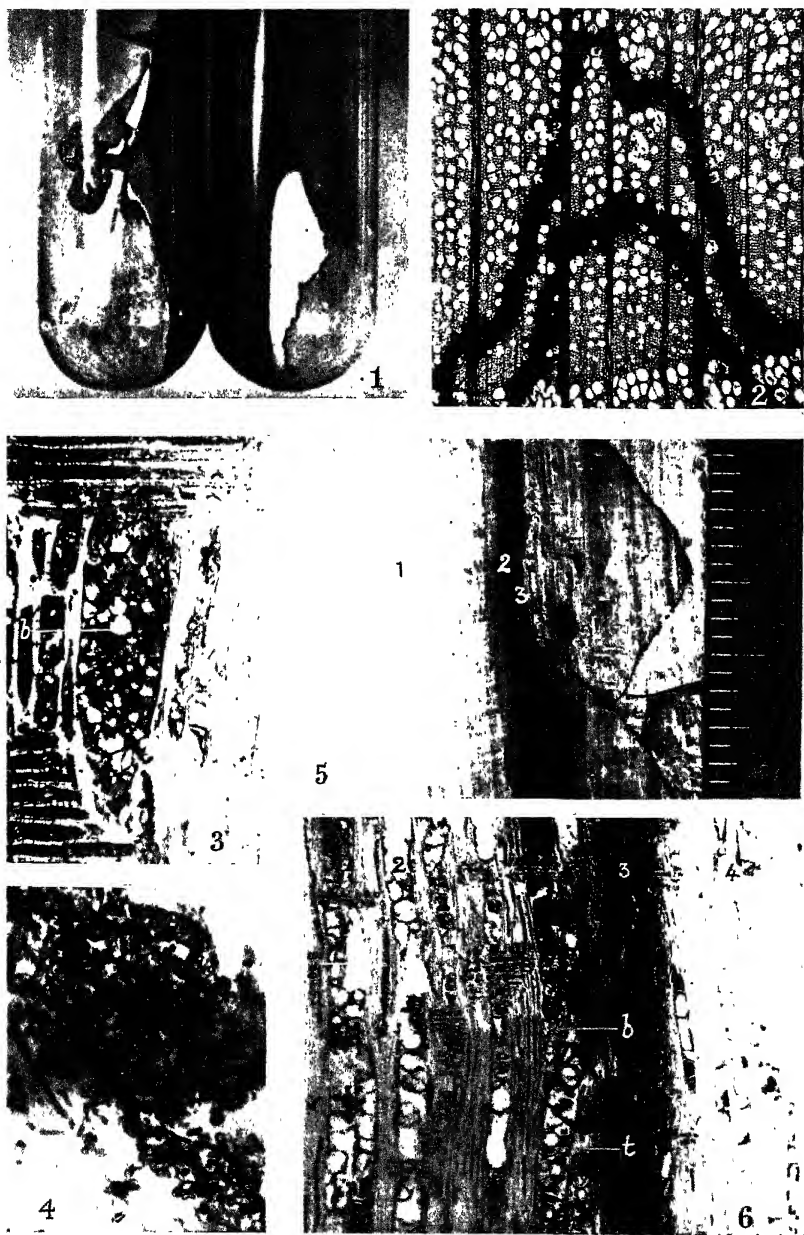
Fig. 2.



Fig. 1.



CAMPBELL.—ZONE LINES IN PLANT TISSUES (pp. 123-145).



CAMPBELL.—ZONE LINES IN PLANT TISSUES (pp. 123-145).

- Fig. 3. 7-day culture on sterile carrot block incubated at 25° C. in daylight. The zonation corresponds to the alternate day and night periods. $\times 2$.
- Fig. 4. 10-day culture on sterilised beech wood. $\times 1$.
- Fig. 5. Group of conidial bearing stromata developed on *C.* medium. The dark patches on the stromata are exuded globules of water. $\times 1$.
- Fig. 6. Culture on sterilised lime wood showing a stroma and the black ovoid bodies developed in the cotton-wool at the foot of the tube. $\times 1$.
- Fig. 7. Conidial bearing stromata developed on sterilised elm wood. The growth of the long stroma was checked by dropping the temperature from 25° to 18° C. and in consequence a new growth has arisen just below the checked tip causing the malformation seen in the middle of the stroma. Note the production of hairs on the lower half of the stroma by the fall in temperature. $\times 1$.

PLATE XIII.

- Fig. 1. Two young cultures of *X. polymorpha* on malt agar. On the left can be seen a young stroma pressed against the glass and on the right a "black line," which is a sectional view of the black incrustation on the medium. $\times \frac{3}{2}$.
- Fig. 2. Transverse section of beech wood showing double black lines. The irregular course of the black line with reference to the wood tissues can be followed. $\times 25$.
- Fig. 3. Longitudinal section of black line in beech wood. The vessels can be seen filled with bladder hyphae (*b*), while the thinner hyphae are found in the tracheides and cells of the medullary ray. Note the staining of the pits of the medullary ray by the brown pigment. Unstained. $\times 400$.
- Fig. 4. Section of the black incrustation formed by *X. polymorpha* in culture showing the early stages in the formation of the dark bladder hyphae. Some of the thinner brown hyphae with well-marked septa can be seen. Unstained. $\times 300$.
- Fig. 5. Beech wood attacked by *F. applanatus* shown in longitudinal section. The normal uninfected wood (1) is separated from the typical rot (4) by a band of "wound gum" (2), which is produced as a result of the *F. applanatus* attack. In addition, black lines and ovals of *X. polymorpha* (3) are superimposed upon the *Fomes* rot. $\times 1$. Scale in inches.
- Fig. 6. Longitudinal radial section through the above specimen. No sound wood is shown in this section but the figures 2, 3 and 4 refer to the same areas. The bladder hyphae (*b*) can be seen filling the remaining spaces in the vessels left by the tyloses (*t*), which have become filled with "wound gum." The fractured deposits of "wound gum" (*w.g.*) can be seen filling the lumina of the tracheides. Unstained. $\times 60$.

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THE BIOLOGICAL DECOMPOSITION OF PLANT MATERIALS

PART VIII. THE AVAILABILITY OF THE NITROGEN OF FUNGAL TISSUES

BY A. GEOFFREY NORMAN, M.Sc., Ph.D., F.I.C.,

Rockefeller Fellow in the Biological Sciences.

(From the Department of Agricultural Bacteriology, University of Wisconsin, Madison, Wisconsin, U.S.A.)

(With 2 Text-figures.)

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I. INTRODUCTION.

IN earlier papers of this series certain chemical aspects of the decomposition of plant materials were discussed. While the assimilation of the plant constituents by micro-organisms is the most obvious feature of decomposition, attention has also been directed to the importance of the accompanying synthesis of microbial tissue. It is difficult to form any very exact estimate of the quantity of such microbial tissue synthesised during decomposition; it may vary from 5 to 20 per cent. of the original substance according to the condition and the availability of the material and the type of organism present. As has been pointed out, the supply of available nitrogen to meet the synthetic or structural needs of the micro-organisms is, in nature, not infrequently a limiting factor. The nitrogen is immobilised by the organisms as protein and other nitrogenous compounds in the microbial tissue. This is, of course, the case in farmyard manure and composts. If such materials are subsequently applied to

the soil, the manurial value will depend chiefly on the amount and the rate of liberation of this nitrogen in an inorganic and plant-available form. The organic matter of the soil itself must be, in part, composed of such microbial residues, the availability or stability of which is of prime importance in determining soil fertility. Many investigators have stated that microbial tissue is very resistant to decomposition, and that the nitrogen of fungal and bacterial cells is, accordingly, relatively unavailable. If it were really so, soil microbiological activities would have long since reached a very low level, and the soil would have a high content of organic nitrogen. The only logical deduction is that microbial nitrogen must be available. The point at issue is therefore whether it is liberated rapidly or only very slowly.

It is not proposed to attempt a complete review of the literature dealing with the decomposition of microbial tissue and the availability of its nitrogen, since such has very recently been given by Jensen (4).

Heck (3), in 1929, carried out a detailed study of the decomposition of fungal tissue in the soil. He concluded that such material decomposes readily in moist soils, since from 40 to 60 per cent. of the carbon is liberated as carbon dioxide in 26 days, and from 30 to 40 per cent. of the nitrogen during the same period. Furthermore, he claimed that the nitrogen of fungal tissue is nitrified as rapidly, or perhaps even more rapidly, than that of other organic materials of similar nitrogen content. His results indicate an extremely close correlation between the nitrogen nitrified and the C/N ratio of the tissues employed, a fact which was, perhaps, insufficiently stressed by him. Those tissues with a narrow C/N ratio underwent rapid nitrification, while if the ratio was wide nitrification in the same period was slow. It was unfortunate that in this work Heck largely employed tissues of higher fungi, not involved in general soil processes. While it is probable that such species as *Polyporus* or *Fomes* are similar in composition to the common soil organisms, it is by no means certain. The only soil organisms employed by this worker were *Aspergillus oryzae*, *Trichoderma lignorum*, and *Coprinus radians*. It must be admitted that these particular samples did not differ in behaviour from the others, but, nevertheless, it seemed desirable to extend the investigation to other soil organisms and to pay particular attention to the influence of the C/N ratio of the tissue, as distinct from the availability of the nitrogenous constituents. The major part of the work to be described, therefore, is a study of the nitrification of numerous samples of fungal tissue and the comparison of these with different controls built up to the same C/N ratio.

An important communication by Jensen (4) dealing with the de-

composition of the cells of soil micro-organisms recently appeared. He was particularly concerned with the nitrification of farmyard manure in soil, and therefore with the availability of microbial tissue. He found nitrification of fungal tissue to be quite rapid up to a point, but not complete, and postulated the existence of some part of the nitrogen in a highly resistant form. Heck(3) had found a direct correlation between the C/N ratio of the tissue and the degree of nitrification in equal time, but although Jensen considered this as a possibility he was unable in his results to find any clear relationship between the two. The experiments to be described bear directly on this point of variance and support the conclusions arrived at by Heck(3).

II. SCHEME OF WORK.

Investigations of the availability of the nitrogen of fungal tissue were carried out along three distinct lines.

(i) The suitability of fungal nitrogen as a source of nitrogen for the decomposition of straw was examined, and the extent of decomposition compared with that produced in the presence of various simpler nitrogenous compounds. In most cases, a mixed soil flora was employed to decompose the straw and fungal tissue mixture; but a few experiments were made in which the decomposition was effected by an organism in pure culture.

(ii) The rate of liberation of ammonia from fungal tissue by pure cultures of reputedly active ammonifying bacteria was studied.

(iii) The nitrification in soil of a number of samples of fungus tissue was compared with that of controls of equal C/N ratio, which consisted of cellulose, straw and glucose, each with added inorganic nitrogen, in an attempt to disentangle the two factors affecting nitrification, namely, the effect of the C/N ratio of the material, and the availability of the nitrogenous constituents.

III. EXPERIMENTAL.

(i) *Fungal tissue as a source of nitrogen for the decomposition of straw.*

The fungus tissue employed was obtained by growth in large pans on a glucose-ammonium nitrate medium in the presence of calcium carbonate. In two cases the quantity of the nitrogen source was increased fivefold in order to obtain microbial tissue of high nitrogen content and narrow C/N ratio. The inoculum was a generous suspension of spores washed off on agar slant in a bottle. The pans were left at room temperature for 10 days, and the mycelial pad then killed by brief autoclaving. After

thorough washing to remove calcium carbonate the mycelium was dried gently in a vacuum oven at 60° C. and subsequently ground to a fine powder and sieved till of approximately uniform particle size. The following tissues were employed in this section of the investigation:

	N content %	C content %
*1. <i>Aspergillus versicolor</i>	7.57	41.9
*2. Mixed tissue (fungi and bacteria)	7.18	42.4
3. <i>A. fumigatus</i>	3.40	43.4
4. <i>A. terreus</i>	3.22	39.1
5. <i>A. niger</i>	2.59	41.0

* Grown on high nitrogen medium.

Equal quantities of oat straw (0.79 per cent. N) were placed in small bottles holding about 40 gm. and a source of nitrogen was added at the rate of 0.8 gm. per 100 gm. dry straw. The calculated quantities of the simple nitrogenous sources were added in solution; while the appropriate amounts of the fungal tissues containing an identical quantity of nitrogen were, on the other hand, thoroughly mixed with and dispersed

Table I.

Decomposition of oat straw supplied with different sources of nitrogen.
48 days—30° C.

37.4 gm. dry straw (containing 0.29 gm. N) and additional nitrogenous material containing 0.3 gm. N = 0.59 gm. total N.

No.	Additional nitrogenous source	Weight of N source added gm.	Residue gm.	% decom- position of mixture	% organic N in residue*	Weight of organic N gm.	N not immo- bilised gm.	Added N retained in organic form gm.	N retained per 100 gm. straw
1	None	—	18.98	49.3	1.69	0.32	+0.03	—	—
2	None	—	19.45	48.0	1.75	0.34	+0.05	—	—
3	Amm. carbonate	1.00	14.41	62.5	2.96	0.43	0.16	0.14	0.37
4	Amm. carbonate	1.00	14.34	62.7	2.93	0.42	0.17	0.13	0.35
5	Sodium nitrate	1.82	15.71	60.0	2.83	0.44	0.15	0.15	0.40
6	Urea	0.60	14.56	61.7	3.20	0.47	0.12	0.18	0.48
7	Amm. nitrate	0.80	14.41	62.3	3.84	0.41	0.18	0.12	0.32
8	Peptone	1.83	15.31	61.0	2.80	0.43	0.16	0.14	0.37
9	Sodium nitrite	1.64	16.52	57.7	3.94	0.49	0.10	0.20	0.53
10	<i>A. versicolor</i>	3.97	17.25	58.3	3.13	0.54	0.05	0.25	0.67
11	<i>A. versicolor</i>	3.97	16.62	59.3	3.20	0.53	0.06	0.24	0.64
12	Mixed tissue	4.18	15.32	63.2	3.85	0.59	0.00	0.30	0.80
13	Mixed tissue	4.18	15.30	63.2	3.64	0.56	0.03	0.27	0.72
14	<i>A. fumigatus</i>	8.82	18.75	59.6	3.00	0.56	0.03	0.27	0.72
15	<i>A. fumigatus</i>	8.82	19.30	58.4	3.07	0.59	0.00	0.30	0.80
16	<i>A. terreus</i>	9.31	18.00	61.5	3.34	0.60	+0.01	0.30	0.80
17	<i>A. terreus</i>	9.31	18.45	60.5	3.21	0.59	0.00	0.30	0.80
18	<i>A. niger</i>	11.60	23.07	53.0	2.66	0.60	+0.01	0.30	0.80
19	<i>A. niger</i>	11.60	22.60	53.9	2.63	0.59	0.00	0.30	0.80

* Total N—inorganic N (i.e. $\text{NH}_3\text{—N} + \text{NO}_3\text{—N}$ where present).

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throughout the straw in the dry state. Water was added to give a final moisture content of 80 per cent. on the basis of total organic matter present. No special inoculum was added, since it has been found that straw commonly carries a sufficiently diverse flora for decomposition. The bottles were loosely plugged with cotton-wool and incubated in a horizontal position at 30° C., being turned and stirred frequently during the first few days to ensure complete and even wetting. At the end of 48 days, the contents of the bottles were analysed for ammoniacal and total nitrogen (nitrate also where necessary) and for loss of organic matter. The results are summarised in Table I.

The particular sample of oat straw employed had an unusually high nitrogen content (0.79 per cent.) and without an additional supply of nitrogen lost 49 per cent. organic matter in decomposition. In the presence of ammonium carbonate, however, the nitrogen factor was 0.36, that is, 100 gm. straw in decomposing would immobilise or convert to the organic form an additional 0.36 gm. N and at the same time suffer a loss of over 62 per cent. of the organic matter. Other simple sources of nitrogen appeared to be almost equally suitable, with the exception of nitrite, which had, as might have been expected, a slightly depressing effect on the amount of straw decomposed. When the additional nitrogen required was supplied in the form of fungus tissue, decomposition in all cases was normal. The rotted product was rather darker in colour than usually is the case when inorganic N is supplied, and in addition had an odour as of manure, in contrast to the slightly sweet odour of the decompositions in the presence of inorganic nitrogenous salts. It is a little difficult fairly to compare the figures for percentage decomposition in the presence of fungus tissue with those just mentioned, owing to the presence of additional carbonaceous material in the added tissue. This is very considerable in those cases in which the nitrogen content of the tissue is low. Despite this limitation, however, it may easily be seen that the extent of decomposition is of the same order whether nitrogen is supplied in an inorganic form or in the form of fungus tissue. The data are insufficient to justify a claim that the latter source may be preferable, though the figures obtained in bottles 12, 13, which were supplied with a sample of mixed fungal and bacterial tissue, might indicate this. It is interesting to compare these two bottles with Nos. 10, 11 supplied with *Aspergillus versicolor* tissue. They are quantitatively comparable since the weight of added tissue was almost the same in both cases. The mixed tissue is clearly more suitable as a nitrogen source, and decomposition is 4.4 per cent. greater. Bottles 16, 17 and 14, 15 are also approxi-

mately comparable. In this case, although rather more carbonaceous material was added, the *A. terreus* tissue is shown to be slightly more suitable and decomposable than that of *A. fumigatus*. It is doubtful whether this difference is really significant.

Though in all these experiments with added fungal tissue there is present an excess of nitrogen over the determined requirements of the straw in decomposition, little is lost during the period of the experiment except in the case of the *A. versicolor* tissue. The calculated nitrogen factors in Nos. 10-19 are not really valid inasmuch as the quantity of carbonaceous material present at the beginning of the experiment is greater in these cases owing to the presence of the added mould mycelium. It is, no doubt, this additional carbonaceous material which causes the retention of a higher amount of nitrogen.

In a few experiments, the decomposition was carried in pure culture instead of by the agency of a mixed flora. The procedure was similar to that described earlier, except that all constituents were sterilised. The inoculum in each case was a suspension of the spores of the desired organism obtained by washing off a colony grown on agar. At the close of the experiments platings were made from the decomposed straw to check the purity of the organism responsible for the rotting. Table II gives the results of these experiments.

Table II.

Decomposition of oat straw by pure cultures of fungi. 48 days—30° C.

37.4 gm. dry straw (contains 0.29 gm. N) and additional nitrogenous material containing 0.3 gm. N.

No.	Organism	Additional nitrogenous source	Weight of N source added gm.	Residue gm.	% decom- position of N in mixture	% organic residue	Weight of organic N gm.	N not immo- bilised gm.	Added N	
									retained in organic form gm.	retained per 100 gm. straw
1	<i>Trichoderma</i> sp.	Amn. carbonate	1.00	28.19	26.6	1.55	0.44	0.15	0.15	0.40
2	"	Mixed tissue	4.18	29.69	28.1	1.63	0.49	0.10	0.20	0.53
3	"	<i>A. niger</i>	11.60	36.80	25.0	1.52	0.56	0.08	0.27	0.72
4	<i>A. niger</i>	Amn. carbonate	1.00	33.21	13.6	1.40	0.46	0.13	0.17	0.45
5	"	<i>A. niger</i>	11.60	38.34	21.8	1.36	0.52	0.07	0.23	0.61
6	<i>A. versicolor</i>	Amn. carbonate	1.00	27.66	27.5	1.59	0.44	0.15	0.15	0.40
7	"	Mixed tissue	4.18	28.64	31.1	1.84	0.53	0.06	0.24	0.64
8	<i>A. terreus</i>	Amn. carbonate	1.00	28.60	25.5	1.71	0.49	0.10	0.20	0.53
9	"	Mixed tissue	4.18	26.10	37.1	1.93	0.51	0.08	0.22	0.59
10	"	<i>A. niger</i>	11.60	37.60	23.3	1.56	0.59	—	0.30	0.80

Owing to the different quantities of carbonaceous material added, it is again difficult to make valid comparisons. In each case, however, there was a definitely higher percentage loss of organic matter in the presence of microbial nitrogen than in the presence of ammonium

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carbonate. Since the number of organisms and samples of fungus tissue tested is not very large, it is perhaps unwise to draw any general conclusion. Reviewing, however, these results together with those presented in Table I, the indication is clearly that the nitrogenous constituents of fungal mycelium are far from being resistant, and indeed seem readily available to micro-organisms whether alone or in a mixed flora.

(ii) *Liberation of ammonia from fungal tissue by bacteria.*

It was thought that a more direct test of the availability of fungal nitrogen might be given by determining the ammonification effected by known ammonifying bacteria. Two samples of fungal tissue only were employed, one with a high and the other with a low nitrogen content:

	% N	% C	C/N
(i) <i>A. versicolor</i> tissue	7.57	41.9	5.5
(ii) <i>A. niger</i> tissue	2.59	41.0	15.8

The following bacteria were selected on the basis of reports in the literature as to their activity in ammonification: *B. subtilis* (two strains), *Mycoderma* (from silage), *Proteus mirabilis*, *P. vulgaris*, *B. mesentericus*, *Sarcina lutea*, and *B. fluorescens liquefaciens*. In addition an enrichment culture was obtained from soil by repeated transfers in peptone broth.

The experiments were carried out in sand culture, 100 gm. sand being placed in each of a number of 250 c.c. Erlenmeyer flasks. To each was added 1 gm. of fungus tissue, which was then thoroughly distributed throughout the sand by shaking. After sterilisation each was inoculated with 2.5 c.c. of a 3-day-old culture of the respective bacteria in 1 per cent. peptone broth. In addition 20 c.c. of sterile water containing 1.0 gm. KH_2PO_4 per litre was added to each. The flasks were plugged with cotton-wool and incubated at 30° C. At the end of 1, 2 and 4 weeks samples were taken for analysis. Liberated ammonia was washed out of the sand by leaching on a filter paper with 5 per cent. KCl and determined subsequently by distillation with MgO. Controls were similarly treated in the absence of fungal tissue. Table III summarises the results obtained, due correction having been made for ammonia from the peptone in the inoculum. The two samples of tissue behaved very differently, and only traces of ammonia were liberated from that with a low nitrogen content, and a wide C/N ratio. However, even the *A. versicolor* tissue with a nitrogen content of over 7 per cent. did not exhibit rapid ammonification with any of the organisms employed, 19 per cent. being the maximum found in any case. It is believed that some ammonia was

lost by volatilisation, since nitrogen balances made by determining the organic nitrogen remaining in the sand were a little short. For this reason it is clear that such results do not give a very reliable index of availability of the nitrogen, but are included because of their bearing on the question of the influence of C/N ratio on availability.

Table III.

Ammonification of fungus tissue in sand culture.

1 gm. of tissue taken (*A. versicolor* = 75.7 mg. N, *A. niger* = 25.9 mg. N).

		<i>A. versicolor</i> tissue, C/N = 5.5					
		7 days		14 days		28 days	
No.	Ammonifying organism	mg. $\text{NH}_3\text{--N}$	% total N	mg. $\text{NH}_3\text{--N}$	% total N	mg. $\text{NH}_3\text{--N}$	% total N
1	Mixed soil ammonifiers	4.8	6.3	7.6	10.0	11.9	15.7
2	<i>B. subtilis</i> (i)	6.3	8.3	5.9	7.7	1.9	2.6
3	<i>B. subtilis</i> (ii)	5.3	7.0	8.3	11.1	13.8	18.3
4	<i>Mycoderma</i> (silage)	5.5	7.3	4.7	6.3	6.4	8.4
5	<i>Proteus mirabilis</i>	4.5	6.0	4.7	6.3	12.4	16.4
6	<i>P. vulgaris</i>	6.3	8.3	5.5	7.3	3.5	4.6
7	<i>B. mesentericus</i>	6.1	8.1	4.7	6.3	14.5	19.2
8	<i>Sarcina lutea</i>	5.7	7.5	5.2	6.9	2.2	2.9
9	<i>B. fluorescens liquefaciens</i>	7.5	9.9	8.7	11.5	8.4	11.1

		<i>A. niger</i> tissue, C/N = 15.8					
1	Mixed soil ammonifiers	—	—	—	—	0.4	1.5
2	<i>B. subtilis</i> (i)	—	—	0.6	2.6	0.6	2.3
3	<i>B. subtilis</i> (ii)	—	—	0.9	3.5	1.0	3.9
4	<i>Mycoderma</i> (silage)	0.7	2.7	0.7	2.7	0.6	2.3
5	<i>Proteus mirabilis</i>	—	—	—	—	1.1	4.1
6	<i>P. vulgaris</i>	0.1	0.4	0.9	3.5	1.1	4.1
7	<i>B. mesentericus</i>	0.2	0.8	—	—	1.1	4.1
8	<i>Sarcina lutea</i>	—	—	0.1	0.4	2.5	9.7
9	<i>B. fluorescens liquefaciens</i>	—	—	—	—	—	—

(iii) *Nitrification of fungal tissue in soil.*

Since the ammonification experiments with pure cultures were not successful in throwing much light on the availability of the nitrogen of fungal tissue, nitrification experiments were carried out in soil under conditions likely to be more normal for microbial development. Two main series of experiments were made, one in soil of low organic matter (a surface soil from a hill-side) and the other in a soil rich in organic matter and of high initial nitrate content (from a glasshouse). It was considered that the latter would be a soil of higher microbiological activity than the former, and therefore likely to provide the best conditions for the decomposition of the fungal tissue. The experiments were

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in each case carried out in a mixture of equal weights of sand and soil, so that aeration might be satisfactory and the even distribution of the added tissue more readily effected. A weighed quantity of tissue was added to 100 gm. sand and mixed carefully. On the addition of the necessary amount of air-dry and sieved soil and after thorough shaking, good distribution was achieved. The fungal tissue had previously been killed by brief autoclaving, dried and finely ground till capable of passing through a 40-mesh sieve. It was thought advisable to employ the tissue in this state so that distribution might be satisfactory. The consensus of opinion of other workers is that tissue, fresh, or air dried, but not killed, is more available than when heat killed. This was stated independently by Heck⁽³⁾, by Barthel and Bengtsson⁽¹⁾ and by Engel⁽²⁾. This last worker, in the case of *Azotobacter chroococcum*, compared the rate of nitrification of material prepared in three different ways. Cells dried at 105° C. gave a liberation of 33 per cent. of the total N, living cells scraped off Petri plates and incorporated in the soil gave 44 per cent., and cells actually developed and grown in the soil gave 57 per cent. in equal time. The moisture content was, as far as possible, kept constant throughout; initially 40 c.c. was added to the mixture. Samples were taken for analysis at appropriate periods. Free ammonia and nitrate were the only determinations made.

The following samples of tissue were employed in series I with hill-side soil.

	% C	% N	C/N ratio
1. <i>A. versicolor</i>	41.9	7.57	5.53
2. Mixed organisms	41.0	4.32	9.5
3. <i>A. terreus</i>	39.3	3.22	12.20
4. <i>A. fumigatus</i>	43.4	3.40	12.76
5. <i>A. niger</i>	41.0	2.59	15.8

For purposes of comparison a number of parallel experiments were set up with artificial mixtures of the same carbon/nitrogen ratios as the tissues employed. One group consisted of cellulose + nitrogen (in the form of ammonium sulphate) and the other of straw + nitrogen (in the form of ammonium sulphate). In setting these up due allowance was made for the organic nitrogen already present in the straw (0.79 per cent.). The carbon contents of the cellulose and straw were respectively 44.4 and 45.0 per cent. Both were finely divided and distributed in the sand-soil mixture as carefully as possible and treated precisely in the same fashion as in the fungal tissue experiments. The results of both are given together in Table IV. Since the nitrate liberated from the soil alone was very small in amount, the ammonia and nitrate found must be due to the added fungal material, and it is convenient for purposes of comparison

Table IV.

Nitrification of fungal tissue in hill-side soil.

- (1) *A. versicolor*. C/N=5.53: C=41.9, N=7.57. 1 gm. taken + 100 gm. soil + 100 gm. sand.
Expressed on 1 gm. material (75.7 mg. N).

Period in days	Mould tissue			Cellulose + (NH ₄) ₂ SO ₄			Straw + (NH ₄) ₂ SO ₄		
	NH ₃ —N	NO ₃ —N	% N liberated	NH ₃ —N	NO ₃ —N	% N liberated	NH ₃ —N	NO ₃ —N	% N liberated
48	0.3	45.4	60.4	0.5	56.6	75.5	0.3	34.6	46.0
90	0.3	52.1	69.1	0.4	62.3	82.8	0.2	33.0	43.8
120	0.3	46.4	61.7	1.0	57.4	77.0	0.5	35.0	46.9
180	0.6	61.1	81.4	0.5	58.2	77.5	0.3	33.7	45.0

- (2) Mixed fungi and bacteria. C/N=9.5: C=41.0, N=4.32. 1.3 gm. taken and 100 gm. soil and 100 gm. sand. Expressed on 1 gm. material (43.2 mg. N).

Period in days	Mould tissue			Cellulose + (NH ₄) ₂ SO ₄			Straw + (NH ₄) ₂ SO ₄		
	NH ₃ —N	NO ₃ —N	% N liberated	NH ₃ —N	NO ₃ —N	% N liberated	NH ₃ —N	NO ₃ —N	% N liberated
48	0.2	16.9	39.6	0.3	32.1	75.0	0.1	20.1	46.7
90	0.4	22.7	53.5	0.2	30.9	72.0	0.2	21.3	49.7
120	0.3	21.0	49.3	0.4	33.8	79.1	0.4	22.2	52.3
180	0.6	26.0	61.5	0.3	37.8	88.2	0.4	22.2	52.3

- (3) *A. terreus*. C/N=12.20: C=39.3, N=3.22. 1.5 gm. taken + 100 gm. soil + 100 gm. sand.
Expressed on 1 gm. material (32.2 mg. N).

Period in days	Mould tissue			Cellulose + (NH ₄) ₂ SO ₄			Straw + (NH ₄) ₂ SO ₄		
	NH ₃ —N	NO ₃ —N	% N liberated	NH ₃ —N	NO ₃ —N	% N liberated	NH ₃ —N	NO ₃ —N	% N liberated
48	0.0	10.6	32.9	0.2	27.2	85.0	0.3	15.3	48.5
90	0.2	11.9	37.6	0.3	23.0	72.4	0.1	11.8	37.0
120	—	—	—	0.2	22.7	71.1	0.3	17.9	56.5
180	0.2	16.3	51.2	0.3	23.5	74.0	0.1	19.4	60.5

- (4) *A. fumigatus*. C/N=12.76: C=43.4, N=3.40. 1.5 gm. taken + 100 gm. soil + 100 gm. sand. Expressed on 1 gm. material (34.0 mg. N).

Period in days	Mould tissue			Cellulose + (NH ₄) ₂ SO ₄			Straw + (NH ₄) ₂ SO ₄		
	NH ₃ —N	NO ₃ —N	% N liberated	NH ₃ —N	NO ₃ —N	% N liberated	NH ₃ —N	NO ₃ —N	% N liberated
48	0.2	12.6	37.6	0.4	21.1	63.2	0.2	15.0	44.6
90	0.2	16.0	47.6	0.3	20.5	61.1	0.1	15.1	44.6
120	—	—	—	0.2	20.6	61.1	0.6	17.1	52.0
180	0.3	18.0	53.8	0.4	21.5	64.5	0.3	19.8	59.0

- (5) *A. niger*. C/N=15.8: C=41.0, N=2.59. 2 gm. taken + 100 gm. soil + 100 gm. sand.
Expressed on 1 gm. material (25.9 mg. N).

Period in days	Mould tissue			Cellulose + (NH ₄) ₂ SO ₄			Straw + (NH ₄) ₂ SO ₄		
	NH ₃ —N	NO ₃ —N	% N liberated	NH ₃ —N	NO ₃ —N	% N liberated	NH ₃ —N	NO ₃ —N	% N liberated
48	0.1	5.4	21.2	0.3	13.1	47.9	0.2	10.0	39.4
90	0.2	8.3	32.8	0.1	12.8	49.7	0.3	12.5	49.4
120	0.0	9.4	36.2	0.3	16.7	65.6	0.2	15.8	61.7
180	0.1	10.8	42.0	0.2	15.7	61.4	0.2	15.0	58.7

- (6) Soil. Control. 100 gm. soil + 100 gm. sand. Expressed on 100 gm. soil.

Day	NH ₃ —N	NO ₃ —N
—	Nil	3.1
48	Nil	3.5
90	Nil	3.3
120	Nil	4.0
180	Nil	4.4

to express this as a percentage of the total organic nitrogen added. It will be seen from Fig. 1 that there is a very clear correlation between the C/N ratio of the fungal tissue, and the nitrogen liberated as ammonia and nitrate (this liberation is termed by Jensen and others "mineralisation"). Nitrification of tissue of a narrow C/N ratio (*e.g.* 5.5) was rapid and extensive. 60 per cent. was thus liberated in 48 days and over 80 per cent. in 6 months. At the other end of the scale, a tissue with a much wider ratio, 15.8, was nitrified far less completely in the same period, only 21 per cent. being freed in 48 days, and 42 per cent. in 6 months. On comparing this with the artificially constructed controls

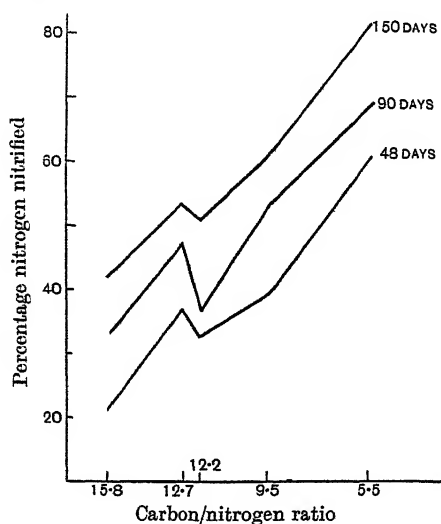


Fig. 1. Summary table of nitrification of fungus tissue in hill-side soil.

of straw and cellulose + N, it seems that nitrification of the fungal tissue is more extensive than that of these mixtures when the C/N ratio is narrow and less so when it is wide. In all cases more nitrogen is liberated from the cellulose controls than from those containing straw. This at first may appear difficult of explanation, and the converse might have been expected since the effective C/N ratio of the straw is undoubtedly narrower than that of cellulose of the same theoretical value, owing to the presence in the former of unavailable carbon in the form of lignin. However, it was observed that the cellulose decomposition was far from complete, and even at the end of the 6 months of the experiment, cellulose fibres were still clearly visible in the soil. This soil was apparently

not biologically very active, and did not carry the necessary flora for the ready and rapid decomposition of all the cellulosic material present. Samples 3 and 4 which have similar nitrogen contents and C/N ratios close together show interesting differences throughout the whole period of the experiment. The *A. terreus* tissue, though with a slightly wider ratio, nitrifies more readily than the *A. fumigatus* tissue. Such minor irregularities as this are to be expected and are explicable on the basis of a different proportion of the various carbonaceous constituents.

The following samples of tissue were employed in series II with glasshouse soil.

	% C	% N	C/N ratio
1. <i>A. ochraceous</i>	48.9	2.83	17.3
2. <i>A. flavipes</i>	48.3	3.04	15.9
3. <i>Pen. chrysogenum</i>	45.4	5.39	11.9
4. <i>A. sydowi</i>	39.8	4.73	8.4
5. <i>A. fischeri</i>	43.8	5.40	8.1
6. <i>A. citrosporae</i>	40.7	5.41	7.5
7. <i>A. oryzae</i>	42.1	6.15	6.8

The soil employed for this series was rich both in organic matter and nitrogen, and contained a considerable quantity of nitrate. Analyses gave the following figures: C = 2.56 per cent., N = 0.29 per cent., C/N ratio = 8.8. In addition to the nitrification experiments on the fungal samples, which were carried out precisely as described in the preceding section, a number of parallel experiments were made in which there were present artificial mixtures of the same C/N ratios as the tissues. These consisted of cellulose + nitrogen (in the form of ammonium phosphate), straw + nitrogen, and glucose + nitrogen. The results of all these are given together in Table V. Since the soil initially contained a considerable quantity of free nitrate, it is not feasible to calculate the nitrate found as a percentage of the original nitrogen added, and accordingly no very accurate figure can be given throughout for the percentage of nitrogen nitrified. Instead each sample must be examined individually and compared with the artificially constructed controls. Considering these first, perhaps, it will be seen that there was little difference in the liberation of inorganic N in any case between the glucose + N, and the cellulose + N series. Glucose is, of course, more readily available, but such was the biological activity of this soil that the cellulose appeared to be utilised to the same extent. Incidentally, it may be noted that in the cellulose + N series, the actual nitrification process was slackened for some reason which is not understood, with the result that considerable amounts of ammonia accumulated even over as long a period as 6 months both with narrow and wide C/N

Table V.

*Nitrification of fungal tissue and mixtures of equal
C/N ratio in glasshouse soil.*

- (1) *A. ochraceous*. C/N=17.3: C=48.9, N=2.83. 2 gm. taken + 100 gm. soil + 100 gm. sand.
Expressed on 1 gm. material (28.3 gm. fungal N) + 21.3 mg. soil nitrate N.

Period in days	Mould tissue		Glucose + amm. phos.		Cellulose + amm. phos.		Straw + amm. phos.	
	NH ₃ -N	NO ₃ -N	NH ₃ -N	NO ₃ -N	NH ₃ -N	NO ₃ -N	NH ₃ -N	NO ₃ -N
30	0.6	19.5	14.5	10.3	9.4	18.1	0.7	26.8
60	1.1	24.6	5.7	16.3	3.8	14.4	1.0	28.9
90	0.7	33.4	5.0	17.8	5.4	11.9	0.6	27.1
180	0.5	26.3	4.7	19.1	3.4	19.2	0.8	30.5

- (2) *A. flavipes*. C/N=15.9: C=48.3, N=3.04. 2 gm. taken + 100 gm. soil + 100 gm. sand.
Expressed on 1 gm. material (30.4 mg. N) + 21.3 mg. soil nitrate N.

Period in days	Mould tissue		Glucose + amm. phos.		Cellulose + amm. phos.		Straw + amm. phos.	
	NH ₃ -N	NO ₃ -N	NH ₃ -N	NO ₃ -N	NH ₃ -N	NO ₃ -N	NH ₃ -N	NO ₃ -N
30	0.9	17.6	1.6	22.4	12.8	14.2	1.9	25.5
60	0.7	28.0	0.8	20.1	7.2	15.5	0.5	27.9
90	1.2	42.8	0.1	22.6	5.9	22.6	0.4	30.6
180	0.9	43.5	0.5	27.2	3.7	19.7	0.2	32.4

- (3) *P. chrysogenum*. C/N=11.9: C=45.4, N=5.39. 1.1 gm. taken + 100 gm. soil + 100 gm. sand.
Expressed on 1 gm. material (53.9 mg. N) + 38.6 mg. soil nitrate N.

Period in days	Mould tissue		Glucose + amm. phos.		Cellulose + amm. phos.		Straw + amm. phos.	
	NH ₃ -N	NO ₃ -N	NH ₃ -N	NO ₃ -N	NH ₃ -N	NO ₃ -N	NH ₃ -N	NO ₃ -N
30	3.5	31.4	0.7	23.3	12.5	21.4	2.9	39.5
60	1.3	48.8	0.6	26.2	8.7	18.8	0.9	34.6
90	1.0	42.6	0.2	28.5	10.5	31.7	1.1	40.1
180	1.3	51.4	0.6	35.5	7.2	29.6	2.5	42.4

- (4) *A. sydowi*. C/N=8.4: C=39.8, N=4.73. 1.3 gm. taken + 100 gm. soil + 100 gm. sand.
Expressed on 1 gm. material (47.3 mg. N) + 32.7 mg. soil nitrate N.

Period in days	Mould tissue		Glucose + amm. phos.		Cellulose + amm. phos.		Straw + amm. phos.	
	NH ₃ -N	NO ₃ -N	NH ₃ -N	NO ₃ -N	NH ₃ -N	NO ₃ -N	NH ₃ -N	NO ₃ -N
30	5.1	45.3	1.0	36.3	24.8	41.8	2.7	59.3
60	2.2	59.2	1.0	42.9	14.2	35.6	1.4	59.5
90	0.9	55.6	0.2	57.4	13.0	41.6	1.3	62.7
180	2.4	52.3	0.6	58.4	13.8	38.2	1.6	56.6

- (5) *A. fischeri*. C/N=8.1: C=43.8, N=5.4. 1.1 gm. taken + 100 gm. soil + 100 gm. sand.
Expressed on 1 gm. material (54 mg. N) + 38.7 mg. soil nitrate N.

Period in days	Mould tissue		Glucose + amm. phos.		Cellulose + amm. phos.		Straw + amm. phos.	
	NH ₃ -N	NO ₃ -N	NH ₃ -N	NO ₃ -N	NH ₃ -N	NO ₃ -N	NH ₃ -N	NO ₃ -N
30	2.0	52.8	1.1	35.6	26.8	43.0	1.6	58.2
60	2.1	69.8	1.7	47.0	12.0	40.5	1.5	58.0
90	2.0	66.2	0.4	57.3	13.2	44.6	0.8	65.0
180	0.8	78.3	0.6	66.6	12.4	46.4	0.5	65.1

Table V (contd.).

- (6) *A. citrosporus*. C/N=7.5: C=40.7, N=5.41. 1.1 gm. taken + 100 gm. soil + 100 gm. sand. Expressed on 1 gm. material (54.1 mg. N) + 38.8 mg. soil nitrate N.

Period in days	Mould tissue		Glucose + amm. phos.		Cellulose + amm. phos.		Straw + amm. phos.	
	NH ₃ -N	NO ₃ -N	NH ₃ -N	NO ₃ -N	NH ₃ -N	NO ₃ -N	NH ₃ -N	NO ₃ -N
30	1.0	65.4	1.2	44.4	28.0	44.2	1.3	76.0
60	1.3	67.5	1.6	50.8	18.4	41.4	1.8	68.5
90	2.4	61.6	5.0	57.7	15.7	54.6	1.6	65.6
180	0.6	67.0	1.6	64.2	19.5	47.3	0.7	68.4

- (7) *A. oryzae*. C/N=6.8: C=42.1, N=6.15. 1 gm. taken + 100 gm. soil + 100 gm. sand. Expressed on 1 gm. material (61.5 mg. N) + 42.6 mg. soil nitrate N.

Period in days	Mould tissue		Glucose + amm. phos.		Cellulose + amm. phos.		Straw + amm. phos.	
	NH ₃ -N	NO ₃ -N	NH ₃ -N	NO ₃ -N	NH ₃ -N	NO ₃ -N	NH ₃ -N	NO ₃ -N
30	1.7	68.7	1.5	44.6	25.7	45.5	21.0	60.7
60	1.8	80.5	1.9	62.8	19.4	52.0	14.6	59.0
90	4.5	55.9	1.3	72.3	18.8	55.2	0.8	84.6
180	1.0	88.3	1.7	68.3	9.3	68.0	4.7	58.6

- (8) Soil control. C/N=8.8: C=2.56, N=0.29. 100 gm. soil + 100 gm. sand. Expressed on 100 gm. soil.

Period in days	NH ₃ -N	NO ₃ -N
—	0.2	42.6
30	0.1	42.3
90	0.3	46.7
180	0.4	48.8

ratios. In no other series was this phenomenon observed. Rather more nitrate was liberated from the straw + N series than from either the glucose or cellulose + N series when the C/N ratio was wide, but the differences were small when it was narrower than 9. This was as anticipated, since the actual C/N ratio is somewhat closer than the theoretical owing to the presence and distribution of the lignin. Comparing with these the fungal tissue over the whole period of 6 months, it is seen from Fig. 2 that while there is a definite correlation between C/N ratio, or nitrogen content, and the nitrogen nitrified, this is far from being as regular as in the other series, particularly in those samples with a ratio less than 9. This is especially evident in samples 4, 5 and 6 which are very similar in composition. Samples 4 and 5, with approximately the same C/N ratios but different contents of nitrogen, nitrify to a distinctly different degree, sample 5 being much more available. The latter is also more available than sample 6, which has identically the same nitrogen content as sample 5 and an even narrower ratio. These differences are no doubt due to the varying availabilities of the tissue which will be discussed further later. With a wide ratio (17.3), more nitrogen was liberated from

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the fungal material than in the glucose or cellulose + N series, but less than in the straw series. As the ratio gets narrower, however, the fungal nitrogen is freed to an extent greater than in any of the comparative series. This was especially noticeable in that sample with the narrow ratio of 6.8. Although, for the reasons mentioned earlier, it is not possible to give accurate figures for the percentage nitrogen nitrified, some approximation can be attempted. Nitrification appears to increase from about 20 to 75 per cent. as the C/N ratio is narrowed from 17 to 7,

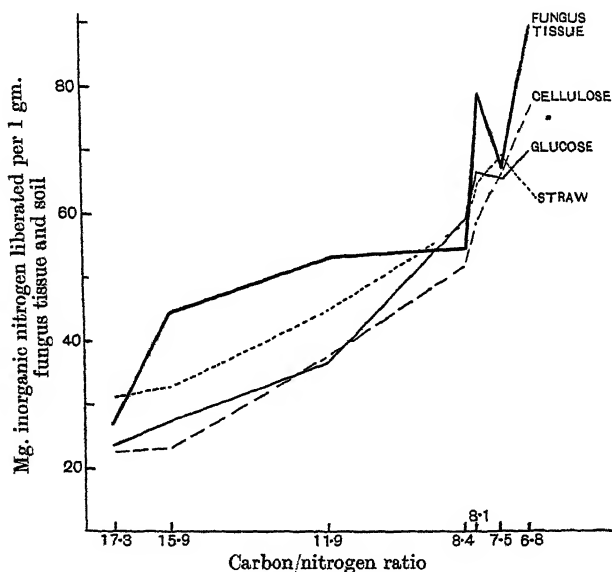


Fig. 2. Summary table of nitrification of fungus tissue and mixtures of equal C/N ratio in glasshouse soil (6 months).

and clear evidence is given for a correlation between the C/N ratio and the extent of nitrification.

IV. DISCUSSION.

Studies on the influence of the C/N ratio on decomposition and nitrification involve a consideration of several factors. The C/N ratio, though an apparently simple expression, is apt to be misleading. Decomposition depends on the C/N ratio directly, only in the unusual case of all the carbon and all the nitrogen being readily available. This, however, is in practice an infrequent occurrence, and the actual C/N ratio is at variance with the apparent figure. If a portion of the carbon

is relatively unavailable the true ratio may be considerably narrower. This is usually the case with plant materials, since the presence and distribution of the lignin reduces appreciably the available carbon. On the other hand, if a portion of the nitrogen is unavailable, the effective C/N ratio may be much wider than the apparent figure. This would be the case in such materials as leather or hair, the protein of which is not easily degraded by soil organisms. Accordingly, if an examination is to be made of the availability of the nitrogen of a given material by comparison with the nitrogen nitrified from identical experiments with the same C/N ratio, the relative availabilities of the carbonaceous constituents have also to be taken into account lest misleading results be obtained owing to the fact that the true and effective C/N ratio differs from the absolute figures upon which the control experiments were based. This is particularly the case when working with a material such as fungal tissue. Very little is known as to the chemistry and practically nothing as to the availabilities of the various individual constituents. The major part of the nitrogen has been shown to be present as soluble protein, and it is probable that such protein differs little from ordinary proteins in availability. In addition, fungal tissue is known to give 10–15 per cent. of an alkali-resistant residue, containing nitrogen, and usually described as fungal "chitin." This chitin has been shown to yield amino-glucose on acid hydrolysis. Its nitrogen content, however, varies usually between 3 and 4 per cent., according to the method of preparation, and this will account for only 33–45 per cent. of the whole residue in terms of amino-glucose. Several workers have suggested that fungal chitin would be very resistant to the attack of micro-organisms, but Jensen (4) has recently proved conclusively that it is readily available. He obtained a liberation of 63·5 per cent. of its nitrogen as nitrate in 4 months. Since, therefore, it would seem that both the types of nitrogenous constituents known to be present are likely to be available, the rate of mineralisation of fungal nitrogen probably depends more on the amount and kind of energy material present. In other words, the availability and rate of decomposition of the carbohydrate constituents will determine the quantity of nitrogen liberated in the inorganic condition. Precise information as to the nature of the polysaccharides of fungi is lacking. The literature contains numerous references to such materials under many names, "Mould starch," "mycodextrins," "para-isodextrin," "spore starch," etc., but neither the constitution nor the availability has ever been determined. The fat and lipid content of fungi varies very considerably, and in some cases amounts to as much as

20 per cent. The distribution of such material might profoundly influence the rapidity of attack on the other constituents, though experiments on one sample of tissue which had undergone extraction with alcohol and saponification with alcoholic potash, did not lend much support to such a view.

The first experiments herein recorded on the availability of fungal nitrogen, and the later ones on its nitrification in the soil, do not indicate any unusual features in the decomposition of this type of material. Certainly they do not substantiate the claims made by Löhnis(5) and others that fungal tissue is very resistant. Rather do they confirm the opinion of Heck(3) and Jensen(4) that the nitrogen is, at least in part, readily available to micro-organisms. Very strong support is afforded to the conclusions of Heck(3) that the rate of nitrification depends on the quantity of energy material available, and approximately expressed by the C/N ratio. This correlation is not absolute, and appreciable differences in behaviour were observed in samples of very similar C/N ratio. It seems that Jensen(4) overlooked this correlation owing to the fact that, with one exception, the samples of microbial tissue employed by him for nitrogen studies had C/N ratios lying between 8.4-12.7. This range is narrow and centred round the point of stability of the soil employed. Even so, a close examination of his results does give an indication of such a relationship though hardly an absolute proof. This is best seen in the experiments carried out by him in sand medium. Of ten samples taken five had a C/N ratio falling between 8.4 and 9.3 with an average of 8.9. The average percentage of nitrogen liberated as ammonia from this group of samples in 90 days was 63.5. Four of the remaining samples had ratios lying between 10.2 and 12.7 with an average of 11.7. From these there was liberated as ammonia only 45.4 per cent. of the nitrogen, a figure significantly lower as would be expected from a group with a wider ratio.

The various controls of equal theoretical C/N ratio employed in this work yielded results which have an important bearing on Jensen's further claim, that a portion of the nitrogen of fungal material persists in the soil as an almost unnitrifiable residue. His statement was based on the fact that the liberation of nitrogen beyond a certain point is slow. He found that little more was freed in 120 days than in the first 60 days. The experiments described in this paper were all continued for a period of 6 months. The highest percentage liberation of nitrogen in that period was 81.4 from a tissue of initial C/N ratio of 5.5. This was actually higher than the recovery from any of the artificial controls to which the nitrogen

was originally supplied as ammonia. Furthermore, in the majority of cases, more nitrogen was mineralised from the fungal tissue than from its corresponding controls. This would indicate therefore that the existence of a resistant residue is somewhat doubtful. It would seem that the ammonification and nitrification processes come practically to a standstill, not because of the unavailability of the remaining material, but because the soil population has reached a position of biological equilibrium. For most soils this condition is attained with a C/N ratio between 10 and 12 and an absence of readily available carbohydrate material. If the initial ratio be narrower, and nitrogen be present in excess of the microbial requirements in decomposition, nitrification will be more rapid than carbon loss and the organic C/N ratio accordingly widened. If the initial ratio is too wide, nitrification will be slight until by the loss of carbon the ratio has been narrowed. The evidence for the existence of a resistant residue is inconclusive, since it may be explained on the basis of the attainment of an equilibrium or of a state in which change is very slow. The fact that the unnitrified portion of the nitrogen varies in amount according to the original C/N ratio supports this thesis.

It is difficult to see why complete nitrification should be expected or, indeed, could take place in such a short space of time. The decompositions which result in the liberation of nitrate are biological, not chemical, and are accomplished by living agents, transforming organic material in their quest for energy, and continually synthesising new tissue as a result of attack on the old. Once a point of biological and nutritional stability has been reached, further nitrification is, in effect, the nitrification of living microbial tissue as and when it dies. Though individual organisms may have only a transient existence, the downgrade process seems to be but slow. Information on this phase of soil transformations is scanty and experiments bearing thereupon would have to be conducted over an extremely long period of time.

V. SUMMARY.

1. Various aspects of the availability of the nitrogen of fungal tissue to micro-organisms have been investigated, with particular reference to the influence of the C/N ratio of the material.

2. Fungal tissue was found to be as suitable a source of nitrogen as ammonium salts and nitrates for the decomposition of straw both by a mixed soil flora and by pure cultures of certain fungi.

3. The liberation of ammonia in sand culture from fungal tissue by pure cultures of reputedly active ammonifiers was measured.

4. The nitrification in soils of a number of samples of fungus tissue was compared with that of artificial mixtures of equal C/N ratio built up from glucose, cellulose and straw, each with added inorganic nitrogen.

5. A very clear correlation was found between the C/N ratio of the fungal material and the nitrogen nitrified. In one series in hill-side soil nitrification increased from 42 to 80 per cent. in 6 months as the ratio decreased from 15.8 to 5.5; in another, from 20 to 75 per cent. as the ratio decreased from 17 to 7.

6. No evidence was found for the existence of a very resistant and unnitrifiable residue from fungal tissue as claimed by some workers, and incomplete nitrification is probably due to the attainment of biological equilibrium or of a state in which change is very slow.

The author is much indebted to E. G. Hastings, Chairman of the Department of Agricultural Bacteriology of the University of Wisconsin, for putting at his disposal the facilities of that Department, and to E. B. Fred and W. H. Peterson for their ready assistance and advice.

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THE NITRIFICATION PROCESS IN SOILS AND BIOLOGICAL FILTERS

By N. W. BARRITT.

(*From the Department of General Microbiology,
Rothamsted Experimental Station.*)

(With Plate XIV.)

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INTRODUCTION.

IN a recent paper on the biological filtration of dilute sucrose solutions the writer (1931) recorded the occurrence of the oxidation of ammonium lactate by means of a culture of bacteria resulting in the simultaneous formation of carbon dioxide and nitrous acid. Though nitrification in a biological filter increases with decrease in soluble organic matter, appreciable amounts of nitrite were recorded in a sectional filter (*loc. cit.*) in regions where the oxidation of soluble organic matter was not complete. It was also noticed that samples of the effluent from the upper sections containing the largest amounts of organic matter, but not showing the presence of nitrite, invariably developed nitrification on incubation, thus indicating the presence of the nitrifying organisms in all sections of the filter. It was then suggested that nitrification probably played a part in the oxidation of organic matter by supplying a hydrogen acceptor

additional to the oxygen of the dissolved air. This theory is supported by consideration of the losses of nitrogen known to occur during the fermentation of organic nitrogen compounds under partially aerobic conditions (Adeney and Letts, 1908; Russell and Richards, 1917).

The occurrence of nitrification in the presence of organic matter has long been a matter of common experience and stands in marked contrast to the conclusions of the classical researches of Winogradsky on the nitrifying bacteria. According to him these organisms require the absence of organic matter and the presence of carbonates, whereas in soil, in filter beds and in river water they may nitrify in the absence of carbonates and in the presence of organic matter. The possibility of a symbiosis existing between the nitrifying organisms and the common heterotrophic organisms has been hinted at by several workers but without any definite data to support it. Though the behaviour of nitrifying organisms in mixed culture has been the starting-point in most investigations of nitrification, it has always been with the object of their ultimate isolation rather than the obtaining of a satisfactory interpretation of the nitrifying process under natural conditions.

In his recent review of the present position of our knowledge of the nitrification process, Winogradsky (1931) deplores the fact that no progress has been made during the last 40 years. A further review of the literature from this standpoint is necessary to reveal in what direction progress is possible and how far it may be achieved without recourse to pure cultures dependent upon a too specialised technique.

HISTORICAL REVIEW.

Pasteur's prediction of the biological nature of nitrification was first fulfilled in 1877 in two independent investigations by Storer in America, and Schloesing and Muntz in France. Schloesing and Muntz (1878) showed that nitrification was not a common property of oxidising organisms, and in 1879 they showed that the process was favoured by a moderate degree of alkalinity and small amounts of organic matter. They also found that all their nitrifying cultures contained one common type of organism in the form of an ovoid micrococcus.

Warington (1878-9) found that nitrification of soil cultures was increased by addition of tartrates which on oxidation yielded a "salifiable base" and also by the addition of chalk which was found to be essential for complete oxidation of the ammonia. His most remarkable, but hitherto neglected, observation was that of a similar stimulating effect of the addition of small amounts (0.005 per cent.) of sugar which

yielded no salifiable base on oxidation. In the absence of both chalk and organic matter no nitrification was possible. In 1885 he showed the existence of an optimum alkalinity. In 1888 and 1891 he recorded the failure of the gelatine plate method of isolation, though his nitrifying cultures contained a particular type of organism common to all, viz. a micrococcus which formed zoogloea.

Heraeus (1886), in contrast to the findings of Schloesing and Muntz, and of Warington, found nitrification to be a common property of several well-known bacteria including common pathogenic types. Though these results have not been definitely confirmed, other later workers have obtained similar results. Frankland accounted for these results by the reduction of nitrates occurring in the water used in making the culture solutions, whilst Winogradsky suspected the absorption of nitrous acid from the air. Celli and Zucco (1886) recorded results similar to those of Heraeus.

Munro (1886) was the first to question the necessity of organic carbon to the nitrifying organisms. He found that nitrification could occur to some extent in filtered river water.

Frankland, P. F. and G. C. (1890), definitely proved that organic carbon is not essential to the organism. Since all gelatine plate cultures refused to nitrify, they resorted to isolation of the organism by repeated dilution cultures in mineral salt solution and were ultimately successful in isolating an organism which nitrified ammonia readily and refused to grow on gelatine and peptone media. This organism was a micrococcus or short bacillus similar to that described by Schloesing and Muntz, and Warington. In view of this it is remarkable that fuller recognition of the Franklands' priority should not have been accorded by subsequent workers.

One month after the appearance of the Franklands' paper, Winogradsky (1890) published his account of the isolation of the nitrifying organism. His method was to take "clots" of the zoogloea and magnesium carbonate from enrichment cultures, wash them in sterile water and test for purity by growth on gelatine plates. Those inoculations which showed no growth were removed and re-inoculated into ammonium sulphate solutions. In this way cultures of nitrifying organisms were obtained which would not grow on nutrient gelatine. In 1891 Winogradsky devised his silica gel plate method of isolation, the novelty and ingenuity of which immediately captivated and has ever since dominated the minds of most soil bacteriologists. The organism thus isolated resembled the ovoid micrococcus forming zoogloea as described by Schloesing and Muntz, Warington, and the Franklands. Winogradsky, however, was the

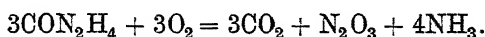
first to describe its metabolic peculiarity of being able to assimilate carbon dioxide in a manner similar to that of his other autotrophic bacteria which derive their energy from the oxidation of sulphur and iron. Not only did he find organic matter unnecessary to this organism but positively harmful. In conjunction with Omeliansky (1899), he found that glucose, peptone and bouillon, at concentrations of 0.2 per cent. and higher, completely inhibited nitrification, whereas glycerine, asparagin, acetates, butyrates and urea only delayed the process. Realising the difficulties of the original silica gel plate for the isolation and cultivation of this organism, in 1925 he published a new method of preparing the plate by adding the mineral salts and ammonium sulphate to the surface of the solid gel followed by a final surface layer of chalk. Inoculations of the organisms are then made on the layer of chalk, and their growth is indicated by the appearance of clear colonies against the white background of undissolved chalk.

Jordan and Richards (1890) found the organism in tap water and in natural waters throughout the state of Massachusetts. On filter beds they found nitrification inversely proportional to bacterial numbers and gelatine plate cultures refused to nitrify. They repeated and confirmed Frankland's dilution method for the isolation of the organism.

Since 1890 many workers have investigated this subject. The autotrophic nature and bouillon sterility of the organisms rigidly described by Winogradsky have been confirmed by Godlewski (1896), Omeliansky (1899), Boullanger and Massol (1903), Löhnis (1904), Joshi (1915), Meyerhof (1916), Bonazzi (1919), Gibbs (1919), Rubentschik (1929), Engel (1930) and Nelson (1931). Calmette (1905) discussed the work of Boullanger and Massol in relation to nitrification in biological filtration. Although excess of ammonia retarded the growth of *Nitrobacter* in pure culture it did not impair its oxidising power. In an established filter the symbiosis between the *Nitrosomonas* and *Nitrobacter* organisms enables large amounts of ammonia to be oxidised direct to nitrates without the appearance of more than traces of nitrites. Similarly he suggested that the presence of organic matter whilst retarding the growth of *Nitrosomonas* does not prevent the oxidation of ammonia by organisms already established. In this way the oxidation of albuminoids to ammonia, of ammonia to nitrite and nitrite to nitrate may occur simultaneously in the same portion of the filter.

Many more workers, however, have obtained results not conforming to the strict requirements of autotrophism. Thus, Leone and Magnanini (1891) obtained the complete nitrification of gelatin; Adeney (1908), in

experiments on the oxidation of peaty matters, found that ammonia and oxygen may disappear without formation of nitrites or nitrates, but on further aeration oxidised nitrogen may be formed in amounts greater than can be accounted for either by the oxidation of ammonia or the reduction of oxygen and concludes that not only the organic nitrogen but also the combined oxygen of the peat takes part in the fermentative changes. He also records the direct oxidation of urea to nitrous acid and gives the equation in support of it:



Beddies (1899) found that growth on silica gel plates was increased by the presence of humus; Stutzer (1901) found agar plates more suitable than silica gel; Fremlin (1903, 1930) obtained the same organism from silica gel plates and broth-agar plates; Chick (1905) isolated nitrifying organisms from sewage by the silica-plate method, but when sterile on bouillon they showed feeble nitrifying powers; Muntz and Lainé (1906) found that the residues of organic decomposition were not harmful to nitrification and that ammonium humate nitrified more rapidly than the sulphate; Coleman (1908) found that addition of dextrose up to 0.2 per cent. increased nitrification in soil, but delayed or suppressed nitrification in pure cultures in sand and in solution. Though the cultures were sterile on bouillon, dextrose was decomposed. He considers that dextrose was not toxic but acted as a stimulant. Owen (1908) obtained better growth of nitrifying organisms on washed agar than on silica gel; Makrinoff (1909) found that gypsum plates were more suitable for isolation and that the presence of soil organic matter was toxic only in large quantities; Beijerinck (1914) criticised autotrophism as applied to *Nitrobacter* (the nitrate-forming organism), since he found it capable of growth on bouillon; Gowda (1924) found it impossible to obtain nitrifying bacteria that would not grow on bouillon; Sack (1925) isolated nitrifying organisms from silica plates which grew better on agar and organic media and could assimilate carbon from both carbon dioxide and sugar. These organisms also oxidised nitrites to nitrates.

A third group of workers has isolated several strains of organisms that nitrify in solution containing organic matter and refuse to grow in inorganic media. Thus, Mischustin (1926) isolated two such organisms from soil; Runov (1926) confirmed this and concluded that nitrification is a common property of many organisms; Cutler and Mukerji (1931) isolated four species of soil bacteria which nitrified ammonium salts in the presence of sugar, but also showed a stronger tendency to decompose

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nitrites. These observations are similar to those made by Heraeus in 1886. Quite recently Lipman and Greenberg (1932) report the isolation of nitrifying organisms capable of oxidising paraffin and of assimilating carbon from this substance.

It would appear from this review that amongst recent workers there exists considerable support for the views of those who worked prior to Winogradsky's clear-cut account of the autotrophic character of the organisms. The condemnation of all workers who record growth of nitrifying bacteria in organic media is somewhat arbitrary and has no *a priori* justification. Such workers may or may not be working with pure cultures, and this point cannot be decided by a negative characteristic which may mean nothing more than damage to the particular inoculation.

In the following experiments no attempt was made to isolate the nitrifying bacteria in a state of purity. A stock culture of nitrifying bacteria was obtained by inoculation into Omeliansky solution of nitrifying material from a biological filter. Inoculations from this stock culture were effected into sterile media by the usual bacteriological technique.

EXPERIMENTAL.

I. THE OCCURRENCE OF NITRIFYING ORGANISMS IN A SECTIONAL BIOLOGICAL FILTER.

(a) *Growth in mineral solutions.*

The sectional filter used in this experiment is described in detail by the writer elsewhere (Barritt, 1931). Inoculations of 1 c.c. of effluent from each section were made with 50 c.c. of sterile Omeliansky solution contained in 250 c.c. conical flasks. After incubation at 22° C. for 14 days the solutions were tested for nitrites colorimetrically by the Griess-Ilosvay reagents with the following results:

Table I.

Sectional culture No.	Nitrite N in parts per million produced in 14 days
1	10
2	15
3	60
4	120
5	120
6	110

Although nitrite was absent from the effluents of sections 1, 2 and 3 it appeared in the cultures in definite amounts and thus confirms the presence of nitrifying organisms in all sections of the filter.

Ashby (1904) used the amounts of nitrite produced in a given time as a measure of the nitrifying powers of soil samples.

Inoculations of 1 c.c. from culture 4 alone into six flasks containing 50 c.c. of Omeliansky solution gave the following results:

Table II.

Flask	Nitrite N in parts per million produced in 14 days
A (1 c.c.)	110
B "	100
C "	100
D "	105
E "	100
F "	110
G (2 c.c.)	190

The amounts of nitrite produced are proportional to the amount of the inoculum with a fluctuation of ± 5 per cent.

The figures in Table I may therefore be considered to be proportional to the respective numbers of nitrifying organisms in the effluents of the various sections of the filter.

(b) *Growth on silica gel plates.*

Silica gel plates were made by Winogradsky's later method (1925). In this method the nutrient salts and chalk are added to the surface of the solidified gel. Plates were inoculated from the sectional filter by means of a platinum loop. After incubation for 14 days the growth of the nitrifying organism was indicated by formation of clear spots in the white layer of chalk. In the centre of these spots colonies of the organism appeared as a raised gelatinous mass of a light brown colour. The rate of growth of the colonies varied with the particular inoculation, those from sections 4, 5 and 6 being more rapid than those from the upper sections (see Plate XIV).

It must be concluded from these experiments that nitrifying organisms occur in all sections of a biological filter and that these organisms are capable of growth under purely autotrophic conditions similar to the *Nitrosomonas* described by Winogradsky. They also live under the heterotrophic conditions of a biological filter supplied with a solution containing 0.2 per cent. sucrose and 0.05 per cent. ammonium sulphate.

II. THERMAL DEATH-POINT OF THE NITRIFYING ORGANISMS.

A series of cultures was set up in Omeliansky solutions. After 4 days, when active nitrification was indicated, the flasks were heated in a water bath for 15 min. at temperatures ranging from 45 to 70° C. It was found that after 14 days no further nitrification had occurred in the flasks

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heated to 58° C., but it continued vigorously in flasks heated to temperatures below 54° C. The thermal death-point of these nitrifying organisms is therefore between 54° and 58° C., which is in agreement with the results of other workers.

III. NITRIFICATION IN SOILS.

A number of soil samples from widely different sources was air-dried in the laboratory and passed through a 1 mm. sieve. Half gram samples of each soil were then put into 50 c.c. of Omeliansky solution and incubated at 25° C. The amounts of nitrite produced were determined at intervals and the results obtained are given in Table III.

Table III.

Soil used for inoculation	Amounts of nitrite N in parts per million after			
	7	14	28	56 days
1. Rothamsted Park Grass AmSO_4 + lime	6	12	40	250
2. " " " AmSO_4 alone	0	0	0*	25
3. " Broadbalk unmanured	0	1	5	300
4. " " dunged plot	45	120	300	Trace (N_2O_5)
5. " " AmSO_4	50	120	300	Trace (N_2O_5)
6. " Agdell	20	65	250	3 (N_2O_5)
7. Woburn	1	20	40	0 (N_2O_5)
8. Harpenden Common (sod)	Trace	1	10	Trace (N_2O_5)
9. " " (worm casts)	3	100	150	0 (N_2O_5)
10. Russian Light Podsol	0	Trace	50	300
11. Nigerian soil (pH 4.1)	0	0	0*	28
12. " " (pH 4.3)	0	0	0*	25
13. " " (pH 8.0)	5	100	300	130 (N_2O_5)

* These soils were inoculated with 1 c.c. of solution from culture 4 on this date.

These results show great differences in the nitrifying powers of different soils. Samples 4 and 5 show the highest rates of nitrification. They represent Broadbalk Plots 2 (dung) and 8 (ammonium sulphate) which have received 87 annual dressings of 129 lb. of nitrogen per acre. Sample 3 came from the Broadbalk unmanured plot. Samples 2, 11 and 12 showed no sign of nitrification after 28 days, and were therefore inoculated with nitrifying organisms from culture 4 to test for the possible presence of a substance inhibitive of nitrification. Nitrification occurred in all three cases, showing that no such substance was present. Sample 2 represents the permanent grass plot which has received ammonium sulphate for 75 years. The pH of this soil is 4.4, due to the accumulation of sulphuric acid. Sample 1 came from the portion of the same plot which had received a dressing of lime every 4 years, the pH of this soil being 6.0.

Samples 8 and 9 are interesting as showing a greater nitrifying power of soil after passage through earthworms. Two factors may account for this increased nitrifying power of worm casts, viz. (1) the removal of the organic matter by digestion by the worm, and (2) the neutralising effect of the calcareous glands of the worm and the possible addition of calcium carbonate from subsoil chalk. Samples 1 and 8, both taken from grass-land, show delayed nitrification and incomplete development. These samples are characterised by the presence of oxidisable organic matter which probably interfered with the normal course of nitrification as shown by samples 3, 4, 5 and 6.

IV. NITRIFICATION IN SOLUTIONS IN THE PRESENCE OF ORGANIC MATTER.

Nitrifying cultures were prepared from the stock culture using Omeliansky solution in which the calcium carbonate was replaced by various organic compounds. The progress of nitrification was tested at intervals. The results obtained for the various substances are given in Table IV.

Table IV.

	Culture solution	Nitrite N in parts per million after					
		3	7	14	21	28	56 days
1.	Mineral salts + CaCO_3	2	50	120	270	400	400
2.	" " + NaHCO_3	0.5	5	70	200	350	0
3.	" " alone	1	15	18	10	Trace	0
4.	" " + urea 0.05 %	0.5	15	45	80	150	0
5 ₁	" " + urea 0.1 %	0.5	4	15	45	80	250
6.	" " + glycine 0.05 %	Trace	1	10	50	120	50
7.	" " + glycine 0.1 %	Trace	—	Trace	1	5	150
8.	" " + uric acid 0.05 %	—	2	35	50	100	200
9.	" " + " " 0.1 %	—	0.5	20	50	130	300
10.	" " + lemco 0.05 %	—	Trace	15	30	45	Nil
11.	" " + " " 0.1 %	—	—	2	20	60	Nil
12.	" " + peptone 0.05 %	—	—	2	30	50	Nil
13.	" " + asparagin 0.05 %	Trace	4	20	20	60	Nil
14.	" " + amm. acetate 0.05 %	—	Trace	10	45	65	50
15.	" " + amm. lactate 0.05 %	—	1	25	40	50	Nil
16.	" " + dextrose 0.05 %	—	Trace	8	20	10	Nil
17.	" " + cellulose 0.05 %	—	Trace	2	50	30	10
18.	" " + paraffin 0.02 %	—	Trace	1	3	20	5

Formation of nitrate occurred in all these cultures.

The course of the nitrification process shows considerable variation with the different organic compounds. Culture 1 with no organic matter and excess chalk shows the most complete nitrification. The buffering effect of the chalk enabled the process to continue uninterruptedly, until all the ammonia was oxidised. In all the other cultures considerable change in pH occurred after 21 days. Ammonification of the organic compounds tended to raise the pH during the first 2 weeks and subsequent

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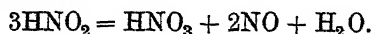
nitrification lowered the *pH* which reached values as low as 5.5 in 28 days and 4.5 in 56 days.

In every case the presence of organic matter tended to retard nitrification as compared with the presence of chalk, the effect in general increasing with the rise in concentration of the carbon and hydrogen content of the molecule. Thus, asparagin, peptone, acetate, dextrose, cellulose and paraffin delayed nitrification in that order and more than did uric acid and urea. This effect may be due to localised anaerobic conditions brought about by the oxidation of these compounds.

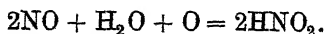
Culture 3, which contained neither chalk nor organic matter, started with an initial *pH* of 7.3 and rapidly became acid, with the result that nitrification ceased after 14 days. In the other cultures without chalk but containing organic matter nitrification proceeded much further. Organic matter appears therefore to take the place of mineral carbonates in supplying the necessary carbon dioxide for synthesis and ammonium carbonate as a temporary buffer. This property of organic matter in promoting nitrification in the absence of mineral carbonates accounts for the stimulating effect of small quantities of sugar observed by Warrington and Coleman and for the observations of Fred and Graul (1916) that in acid soils organic nitrogen nitrifies more rapidly than ammonium sulphate.

When the *pH* falls to 5.5 nitrification ceases followed by disappearance of the nitrous acid, the formation of nitric acid, and a further lowering of *pH* to 4.5. This formation of nitrate before all the ammonia was nitrified was characteristic of most of the cultures especially those in which ammonification was absent, viz. dextrose, cellulose and paraffin.

As a biological process the conversion of nitrous acid to nitric acid at a *pH* of 4.5 appears improbable. It can be accounted for by purely chemical catalysis, by the reactions:



The nitric oxide in solution absorbing oxygen from the air renders the reaction continuous:



Confirmation of this reaction was obtained by acidifying two solutions of sodium nitrite, one with sulphuric acid and the other with acetic acid to *pH* of 5.0. The solution contained 50 parts of nitrite N per million, and after standing in conical flasks at 18° C. for 3 weeks the solutions gave the following results on analysis:

	Parts per million	
	Nitrite N	Nitric N
Acid		
Sulphuric	Nil	47
Acetic	0.5	49

The above reactions probably account for the formation of nitrates in acid soils and in the absence of chalk. They may also account for the observations of Sack (1925) and Lipman and Greenberg (1932) of nitrifying organisms capable of oxidising ammonia direct to nitrates in the presence of dextrose and paraffin respectively. They may also explain the supposed induced oxidation of sodium nitrite by air in the presence of ferrous hydroxide observed by Chakravarti and Dhar (1930) to occur only when alkali is added *after* the ferrous sulphate. The addition of the acid ferrous salt liberates free nitrous acid which would undergo oxidation before addition of the alkali. The formation of ferrous hydroxide is not therefore essential to the oxidation of the nitrite, and the amounts of nitrite oxidised will depend upon the interval of time between adding the ferrous sulphate and its neutralisation by the alkali.

V. INFLUENCE OF REACTION (*pH*) ON NITRIFICATION.

This factor was first investigated by Meyerhof (1916) and later by Meek and Lipman (1922), who found the optimum conditions for the oxidation of ammonia to be between *pH* 7.5 and 8.5. Accurate figures are not easily obtainable, since it is impossible to maintain precise conditions above *pH* 7.0 during active nitrification or below 6.5 in the presence of chalk.

In the absence of chalk it has already been shown (section IV) that the lower limit for nitrification is about *pH* 5.5. Series of Omeliansky solutions containing chalk were prepared with *pH* ranging from 6.7 to 9.2 and were inoculated with 1 c.c. of the stock culture. The amounts of nitrites formed and resulting changes in *pH* after 3 and 7 days are given in Table V.

Table V.
Influence of pH on nitrification.

Period in days	Nitrite N in parts per million													
	1		2		3		4		5		6		7	
	<i>pH</i>	<i>NO</i> ₂	<i>pH</i>	<i>NO</i> ₂	<i>pH</i>	<i>NO</i> ₂	<i>pH</i>	<i>NO</i> ₂	<i>pH</i>	<i>NO</i> ₂	<i>pH</i>	<i>NO</i> ₂	<i>pH</i>	<i>NO</i> ₂
—	6.7	1	7.3	1	7.5	1	8.0	1	8.5	1	8.8	1	9.2	1
3	6.7	3	7.1	3	7.4	3	7.9	3.5	8.5	2.0	8.8	1.5	9.2	1
7	6.7	18	7.0	18	7.1	18	7.6	18	8.4	6.0	8.7	4.0	9.2	1.5

The *pH* was determined colorimetrically using brom-thymol-blue and cresol red indicators. At *pH* 8.5 there is definite reduction in the rate of nitrification, which practically ceases at 9.2. There appears to be no significant difference in the rate of nitrification between 6.7 and 8.0.

VI. NATURE OF THE INHIBITIVE ACTION OF ORGANIC MATTER
ON NITRIFICATION.

It is unlikely that substances like peptone, asparagin and dextrose which are products of organic metabolism would be toxic to any type of bacteria. Their action in depressing nitrification is more easily explicable as being due to the growth of heterotrophic bacteria present in the cultures. Nelson (1931) claims that his pure cultures of *Nitrosomonas* were capable of nitrification in the presence of dextrose in amounts as high as 10 per cent., from which it may be concluded that dextrose is toxic only when the nitrifying culture is impure and that the toxicity is due to the metabolic products of the contaminants.

The recovery of nitrification after 14 days as seen in Table IV suggests that the toxic substance is either unstable or volatile. It was therefore decided to investigate the effect of carbon dioxide on nitrification. Godlewski (1896), Joshi (1915), Bonazzi (1921), and Gowda (1924) found that increasing the carbon dioxide in the air of the culture flasks to 50 per cent. resulted in a slight increase in the amount of nitrite formed especially in the presence of chalk. This result is rather surprising, since the increase in carbon dioxide involved a corresponding reduction in the concentration of oxygen which many workers (Löhnsis, Bonazzi and Berthel) have found to be an important factor in determining the rate of nitrification. Joshi accounts for the observed stimulation as being due to an increased solubility of the calcium carbonate in the culture solution. This implies that the amount of soluble carbonate was a limiting factor, which is very improbable, since ammonium carbonate is formed by interaction between ammonium sulphate and chalk, and the ratio of carbon assimilated to nitrogen oxidised is only 1/35. Further, as nitrification proceeds, the buffering action of the chalk must result in an increasing formation of soluble bicarbonate, which, being indirectly proportional to the amount of nitrite produced, would supply the needs of the organisms more than thirty times over. Some other explanation must be found for this stimulating effect of carbon dioxide observed by these workers.

In the sterilisation of the mineral salt solution interaction between the various salts occurs. Potassium carbonate is formed by interaction between chalk or magnesium carbonate and di-potassium phosphate. Ammonium carbonate is similarly formed from the ammonium sulphate during incubation, especially at the higher laboratory temperatures common in India. The formation of these alkali carbonates results in an

increase in *pH* which at 8.5 retards nitrification. The addition of carbon dioxide to such a culture solution would, by reducing the *pH* nearer to 8.0, result in increased nitrification.

By using culture solutions in which nitrification is already established and in which the possible fluctuations in *pH* are within the range 7.0–8.0 and therefore indifferent, the effect of carbon dioxide concentration *per se* can be more correctly determined. The addition to the cultures of carbon dioxide in the form of a prepared solution of carbonic acid, instead of the maintenance of an increased percentage of carbon dioxide in the air of the culture flasks, more nearly corresponds to the conditions of a solution in which carbon dioxide is being evolved by the fermentation of organic matter under normal atmospheric conditions.

A solution of carbonic acid was obtained from a sparklet siphon. Titration of this solution with *N*/10 soda after standing 1 hour showed it to contain 0.13 per cent. by weight of carbon dioxide.

Various amounts of this carbonic acid solution were added to culture solutions in which nitrification had already proceeded for 7 days, and the subsequent nitrite formation was determined. The results are given in Table VI.

Table VI.

Effect of carbon dioxide on nitrification.

Culture No.	Carbonic acid added gm.	Nitrite N in parts per million				
		At start	After 1 day	After 3 days	After 5 days	After 14 days
1	—	150	180	210	240	400
2	0.006	150	180	210	240	400
3	0.012	150	150	170	190	390
4	0.018	150	140	145	155	380

These results show a definite initial depression of nitrification by the addition of 0.012 gm. of carbon dioxide to 50 c.c. of culture solution, whilst the addition of 0.018 gm. of carbon dioxide stops nitrification completely for 3 days, recovery occurring after 5 days.

On the fifth day sub-cultures were made from cultures 1 and 4 by transferring 1 c.c. of the suspension into flasks containing 50 c.c. of sterile Omeliansky solution. The subsequent nitrification in these two flasks proceeded at equal rates, from which it must be concluded that the numbers of active nitrifying organisms in cultures 1 and 4 were equal. It appears therefore that although carbon dioxide suppressed nitrification in culture 4 the growth of the organisms continued unchanged. This fact rules out toxicity and suggests that lack of oxygen produced by the

increased carbon dioxide tension is the more probable cause of the suppression of nitrification. The continued growth of the bacteria in culture 4 without the production of nitrites could only be possible on the supposition that some intermediate compound was formed similar to that postulated by Beesley (1914) to account for the temporary disappearance of nitrogen (ammonia plus nitrite) in the early stages of nitrification.

The depression of nitrification by the addition of 0.024 or 0.036 per cent. of carbon dioxide fully accounts for the observed effects of organic compounds on this process. The fermentation of 0.05 per cent. peptone or dextrose would result in the production of approximately this amount of carbon dioxide, and the addition of larger amounts of organic compounds by their longer period of fermentation would result in a very prolonged suppression of nitrification.

Attempts to diminish the depressing effect of organic matter by vigorous aeration were only partially successful. Increased aeration not only lowers the concentration of carbon dioxide but also lowers the concentration of ammonia which diminishes the rate of nitrification. The complete removal of carbon dioxide by inserting a tube of strong alkali within the culture flask (*vide* Winogradsky, Omeliansky, Godlewski, Bonazzi and Gowda) leaves an accumulation of free ammonia with a consequent rise in pH above the optimum, resulting in a depression of nitrification. This effect of the removal of carbon dioxide from the cultures led these authors to conclude erroneously that gaseous carbon dioxide was essential to nitrification.

VII. INFLUENCE OF ORGANIC MATTER ON THE GROWTH OF NITRIFYING BACTERIA.

The occurrence of nitrification in the presence of organic matter naturally raises the question of its effect on the autotrophic character of the organisms. Is the growth of the nitrifying bacteria in the presence of organic matter entirely autotrophic (*i.e.* organic synthesis from carbon dioxide)?

Unfortunately the only satisfactory measurement of growth applicable to these organisms is the production of nitrites, and this is affected by changes in the concentration of carbon dioxide and oxygen. In section IV it has already been shown that nitrification was most rapid in cultures containing chalk and free from organic matter. In order to obtain comparative measurements of the growth and nitrifying power of the organisms growing in the presence of organic matter, sub-cultures in Omeliansky

solution are necessary so as to exclude fluctuations in carbon dioxide and oxygen concentration.

For this purpose a series of nitrifying cultures was prepared containing different organic compounds. After incubation at 25° C. for 48 hours, when considerable bacterial growth had occurred, inoculations of 1 c.c. were transferred to Omeliansky solution and the rates of nitrification determined. Similar sub-cultures from the original culture into Omeliansky solution were also made 4 weeks later. The results are given in Table VII.

Table VII.

Composition of culture solution	Amounts of nitrite N in parts per million							
	Primary culture			1st sub-culture (after 48 hours)		2nd sub-culture (after 28 days)		
	2 days	7 days	28 days	4 days	21 days	4 days	10 days	21 days
Mineral salts + CaCO ₃ + Am ₂ SO ₄	Trace	55	400	Trace	350	60	125	400
Mineral salts + 0.05 % glycine	—	1	85	—	250	25	115	400
Mineral salts + 0.05 % asparagin	—	3	40	—	350	35	120	400
Mineral salts + 0.05 % peptone	—	—	50	—	380	35	100	380
Mineral salts + 0.05 % dextrose	—	—	20	—	350	4	85	350
Mineral salts + 0.05 % lactose	—	—	12	—	370	20	90	370

The first sub-cultures made after 48 hours show that the depressing effect of the organic matter on the nitrifying bacteria is not carried over into the sub-cultures except perhaps in the case of glycine. In two cases, viz. the cultures containing peptone and lactose, there appears if anything to have been a slight stimulation of the nitrifying organisms. The increase is less than 10 per cent. and according to section I above is scarcely significant.

The second sub-cultures (on the 28th day) taken from the flasks containing organic matter show a definite depression of nitrification on the 4th day especially with dextrose, but a rapid recovery appears by the 10th day, which is complete on the 21st day except in the case of dextrose and lactose.

Although the results are not conclusive regarding the occurrence of heterotrophic growth in nitrifying bacteria, they definitely show an absence of toxicity from 0.05 per cent. asparagin, peptone, dextrose and lactose.

VIII. CONCLUSIONS.

The foregoing investigations confirm the findings of Boullanger and Massol (1903), Chick (1905) and Russell and Bartow (1915) that the nitrifying bacteria in biological filters are identical with those present in soil, *i.e.* they are capable of autotrophic growth and have a low thermal death-point ($56^{\circ}\text{C}.$). Many of the contradictory results of observations of the nitrification process, such as growth in the absence of mineral carbonates, the stimulating and inhibiting effect of organic matter and gaseous carbon dioxide, the uncertain nature of the growth on bouillon tests and the direct production of nitrates from ammonia, can be accounted for by the metabolism of heterotrophic contaminants and by changes in *pH* of the medium.

Gibbs (1919), Gowda (1924) and Nelson (1931) found that repeated sub-culture under autotrophic conditions did not diminish the numbers of heterotrophic bacteria whether in mineral solutions or on silica gel plates. This may be considered strong evidence of a symbiosis between the two types of organisms and would account for the numerous failures to repeat Winogradsky's work in its entirety. It should be pointed out that Winogradsky obtained his isolation by washing his nitrifying inocula (clots) in sterile water and selecting only those showing bouillon sterility. Nelson later got over the difficulty of removing contaminants by suppressing them with copper sulphate and resorting to single cell technique. Both methods are actually dependent upon chance and perseverance for success. Any nitrifying culture obtained by whatever means would, according to Winogradsky, be condemned as impure if it subsequently acquired the power of growth on bouillon.

The Franklands, before Winogradsky's first publication, obtained by selection nitrifying cultures which showed no growth on bouillon. Later generations, however, developed the property of growth on bouillon. Gibbs, Gowda and Nelson obtained similar results.

According to Winogradsky the nitrifying bacteria can grow only by producing nitrite. To this limitation he added that of toxicity of organic compounds. Pure cultures of the organism are thus characterised by a very restricted mode of existence, which may account for the lack of progress in their study to which he referred.

The question of the internal metabolism of these bacteria was not discussed by Winogradsky. The synthesis of sugar or protein implies the existence of an enzyme system similar to that occurring in the protoplasm of plants, and it would be pushing the unique to extreme limits to suppose

such a system to be incapable of katabolic processes. In this connection it may be mentioned that Beijerinck (1890), Radais (1900) and Bristol-Roach (1926) found that pure cultures of green algae though normally autotrophic were also capable of growth on albuminoids and carbohydrates.

Bonazzi (1921) suggested the possibility of carbon dioxide respiration of the autotrophic bacteria to account for nitrification in the absence of free carbon dioxide in the culture, but since free carbon dioxide is not essential to the process this is not evidence of katabolism. In mixed cultures the necessary carbon dioxide is obtainable from the normal respiration of the heterotrophic bacteria and there is strong evidence of a symbiosis between the two types of organisms. Calmette, Boullanger, and Massol suggested that the effect of organic matter was to check the growth, but not the oxidising powers, of the nitrifiers. Since autotrophic organisms derive their energy for growth from the oxidation of ammonia, a reduction in growth would imply a reduction in the rate of oxidation (nitrification). Nelson (1931) found that enrichment cultures did not become enriched with nitrifying organisms, but that the numbers of heterotrophs maintained an almost constant proportion. This suggests that either the heterotrophs lived at the expense of the nitrifiers or that some of the latter were passing into a heterotrophic condition. Beijerinck held this view of the mutability of the nitrifiers, but Winogradsky could not admit this possibility. To the writer a possible way out of this apparent impasse is the observation of heterotrophic mutants acquiring nitrifying powers and becoming autotrophic. This would give one stage of a possible life cycle, and it appears to have occurred in the cultures obtained from the second sub-cultures described in section VII above. The increase in nitrification in these autotrophic cultures is more rapid than that obtained by similar inoculation from one autotrophic culture to another.

A biological filter probably presents the conditions favourable to the occurrence of such a possible life cycle for the nitrifying organisms.

IX. SUMMARY.

1. An extensive review of the literature on nitrification shows that the workers in this subject may be divided into three groups, viz. (a) the Winogradsky or purely autotrophic group, (b) those who include growth in organic media, (c) those who exclude autotrophic growth.

2. The process of nitrification in soils and filter beds is essentially the same and the organisms concerned exhibit the same cultural characteristics, viz. autotrophic growth in purely mineral solution containing carbonates and low thermal death-point (56° C.).

3. In the absence of carbonates heterotrophic organisms can supply the necessary carbon dioxide by the decomposition of organic matter, a process which accounts for nitrification in acid soils and filter beds. In the absence of a salifiable base the oxidation of ammonia is arrested by the formation of free acid and at a pH of 5.5 the nitrous acid is spontaneously oxidised to nitric acid.

4. The inhibitive action of organic matter on nitrification can be accounted for by accumulation of carbon dioxide and ammonia and deficient aeration. Removal of any of these factors results in increased nitrification.

5. Increased nitrifying power of soil after passage through earthworms is recorded and accounted for.

6. The restrictive influence of Winogradsky's ideas on autotrophism are discussed, and the possibility of nitrifying bacteria being a phase in the life cycle of heterotrophic organisms is suggested.

The investigations described in this paper were carried out at Rothamsted Experimental Station as part of the programme of the Water Pollution Research Board of the Department of Scientific and Industrial Research and are published by permission of the Department.

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EXPLANATION OF PLATE XIV.

Silica gel plates inoculated direct from a sectional filter showing development of colonies of nitrifying bacteria. The numbers of the plates correspond to the numbers of the sections of the filter. No. 4 shows the tracks of a small fresh-water crustacean (*Viguerella viguieri*) which frequently occurs in nitrifying effluents.

(Received May 11th, 1932.)

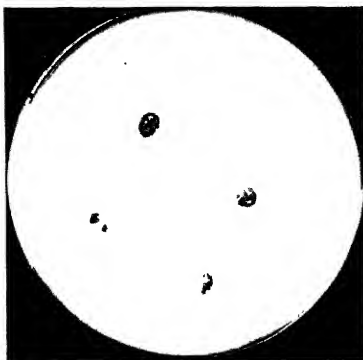


Fig. 1.

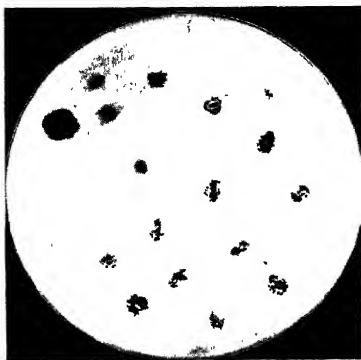


Fig. 2.

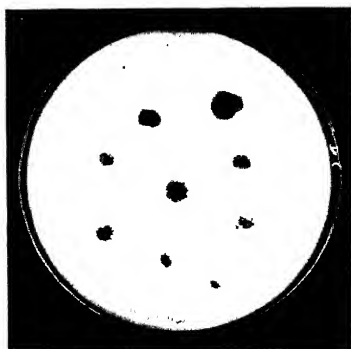


Fig. 3.

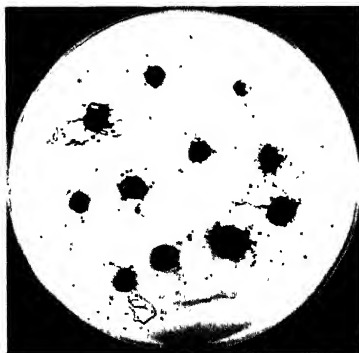


Fig. 4.

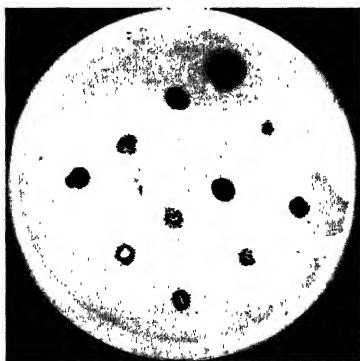


Fig. 5.

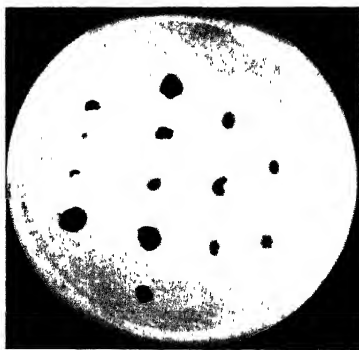


Fig. 6.

DASYNEURA LEGUMINICOLA (LINT.) THE CLOVER SEED MIDGE

BY MARGOT E. METCALFE, PH.D.,

Fellow of the University of Wales.

(*Entomology Department, Rothamsted Experimental Station.*)

(With Plate XV and 2 Text-figures.)

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I. INTRODUCTION.

THE first definite records of the clover seed midge come from America, where Lintner observed the larvae in the heads of red clover in 1877. The midge was described by him as *Cecidomyia trifolii* sp.n. in 1879, but later in the same year he changed the name to *C. leguminicola*. In the *Report on Injurious Insects* 1890, Ormerod (1891) mentions the American clover seed midge, which she states "has only lately appeared in this country—but still we cannot tell with certainty how long it has been here." She goes on to describe the anchor process of the larva, a character distinguishing it from the larvae of *Amblyspatha ormerodi*, another midge found on clover, and there is no doubt that she was actually dealing with *Cecidomyia leguminicola*. The name was again changed, this time by Aldrich in 1905, to *Dasyneura leguminicola*. The midge has also been recorded in Denmark (Schøyen, 1927) and in Wales (Jenkins, 1926).

In 1918 a paper was published by Rockwood and Creel on its control. Barnes (1927) gives a useful summary of the literature to date, and since then Wehrle's paper (1929) dealing with the life history and methods of control has appeared. Apart from Ormerod's notes, the life history of the

clover seed midge, the damage done by the larvae, and the methods of control have not been studied in Great Britain. It is hoped that the following paper will repair this omission. The work was carried out at Harpenden from February, 1931 to July, 1932.¹

II. METHODS.

In order to secure a supply of adults for the purpose of experimentation, over-wintering cocoons of *D. leguminicola* were washed out of the soil adhering to the roots of clover plants brought in from the field in March, 1931. They float on the surface of the water and are easily picked out. They were then placed on damp coconut fibre in jam jars and kept moist.

Material for the second brood was obtained by gathering infested heads of clover from Long Hoos Field, Rothamsted Experimental Station, towards the end of June, when most of the larvae were ready to pupate, but had not left the flower heads. These were placed on damp coconut fibre enclosed by a lamp glass and covered by a circle of muslin sewn to an iron ring. The heads were watered at intervals. Material for the spring brood of 1932 was kept over winter in the same way. In each case a record of the daily emergence of midges and parasites was kept throughout the hatching period.

For the cross-mating experiments, immunity trials, and life-history observations, clover plants growing in pots were covered with muslin bags before the flower heads appeared. Midges were introduced when the heads were at the green stage, and the heads examined for eggs and larvae at suitable intervals. When the larvae were full grown, the heads were cut and sprayed with water at intervals, the larvae which dropped out being placed on sand or damp fibre for pupation.

III. IDENTIFICATION.

Two midges of the genus *Dasyneura*, viz. *D. leguminicola* Lint. and *D. flosculorum* Kieff. (1890), are reported to prevent the formation of seed in red clover. The former is the midge found in America, the latter is recorded from Central Europe (Liebel, 1889; Schlechtendal, 1890), Sweden (Tullgren, 1917), and Great Britain (Bagnall and Harrison, 1918). Kieffer's description of *D. flosculorum* might well be applied to *D. leguminicola*, and for the purpose of distinguishing between the species it was decided to attempt mating experiments. Samples of clover heads infested with *D. leguminicola* had been obtained by Dr H. F. Barnes¹

¹ Dr Barnes very kindly handed over this material and also his MS. notes on this midge when I took over this work on my arrival at Rothamsted in February, 1931.

from Ottawa and Ithaca, in September, 1930. These were kept over winter in coconut fibre in the usual manner. Application was made to Sweden for similar samples of heads with *D. floscolorum*, but proved unsuccessful, and no material was received.

The adults of *D. leguminicola* which hatched in May and June, 1931, were crossed with midges bred from clover in Harpenden. The crosses were successful, mating took place willingly and eggs were laid in every case. In the cross Canadian ♀ × English ♂, adults which appeared to be quite normal were bred through. In other cases larvae were obtained and kept for examination, no attempt being made to rear the adult. The larvae appeared to be normal.

From these experiments, it appears that the clover seed midge present in England is *D. leguminicola* Lint. It still remains to be proved that this is the midge known in Central Europe and Sweden under the name of *D. floscolorum*. *D. leguminicola* bred from English larvae in 1930-2 agrees with Lintner's somewhat meagre description of *Cecidomyia leguminicola*. This has been amplified, and a new description appears below.

DASYNEURA LEGUMINICOLA LINT.

Male. Body length, 1.5-2 mm. Antennae: of typical *Dasyneura* structure; fuscous brown; ranging from 2 + 12 to 2 + 16, the maximum number of individuals having 2 + 14; node of eighth flagellar segment about equal to the neck, about half as long again as broad, slightly constricted towards the middle; neck of third flagellar segment five-sixths of node; terminal node rather longer than preceding, conical; nodes with a regular basal whorl of short setae about half length of the node, a distal irregular whorl of longer setae reaching to about the middle of the succeeding node, and irregular long setae about three times the length of the node. Palps: sparsely haired, pale yellow; 1st and 2nd segments of equal length, 3rd half as long again, distal twice as long as the 2nd; 3rd and distal segments narrower than 1st and 2nd, tapering to the tip. Face: yellow. Eyes: black. Thorax: dorsal region, scutellum, post-scutellum and pleurae dark fuscous, rest of thorax yellow. Wings: hyaline: veins distinctly scaled. Abdomen: deep orange, sterna with anterior and posterior bands of fuscous brown, terga and pleura fuscous brown with patches of dark scales. Legs: pale yellow clothed with dark fuscous scales; claws curved at right angles; empodium slightly longer or same length as claws. Genitalia: basal clasp segment stout with long stout setae; terminal clasp segment curved, stout, with short setae;

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dorsal plate deeply emarginate, the lobes rounded; ventral plate deeply or shallowly emarginate, variable, the lobes narrowly rounded; harpes well developed, digitiform apically; style about the same length or a little longer than the ventral plate.

Described from Cecid. 1864-7 deposited in the Barnes collection.

Female. Body length: rather longer and more robust than in the male. Antennae: of typical *Dasyneura* structure; fuscous brown; ranging from $2 + 12$ to $2 + 16$ with the greatest number of individuals with $2 + 14$; neck of 3rd flagellar segment transverse; segments furnished as in the male with two regular whorls, and irregular scattered setae, shorter than in the male. Palps: light red, 1st segment as long as broad, rather shorter than 2nd segment; 3rd segment about twice as long as basal; terminal segment about twice as long as 2nd. Abdomen: deep red to orange, darker than in the male, intersegmental membranes light, dorsal region with conspicuous bands of dark scales. Ovipositor: pocket type; about twice as long as the body when extended. Otherwise about as in male.

Described from Cecid. 1868-72 deposited in the Barnes collection.

IV. LIFE HISTORY.

According to Wehrle, the clover seed midge is typically two-brooded in the neighbourhood of Ithaca, N.Y., the first brood being on the wing in the early part of June, and the second brood appearing in August and September.

At Harpenden in 1930, the emergence of the first brood started on May 17th and lasted until July 17th, with the crest of emergence on May 30th; in 1931 the first brood hatched between May 27th and June 15th, with the maximum hatch on May 30th and 31st; and in 1932 from May 23rd to June 29th, with the maximum on June 3rd. The second brood in 1931 was on the wing between July 14th and September 19th, with the crest of emergence on August 3rd. In 1932 the first emergences of second brood were on July 15th. In the 1930 first brood the sex ratio was 46 : 54, 1297 males and 1526 females being reared; in the 1931 first brood 65 males and 65 females gave a sex ratio of 50 : 50, and second brood 318 males and 456 females gave a sex ratio of 41 : 59; in 1932 the sex ratio of the 1st brood was 35 : 65, 664 males and 1212 females being reared. The males emerge rather before the females: the dates of the comparative crests of emergence for the two sexes being in 1930 (first brood): males May 28th, females May 30th; 1931 (first brood) males May 28th, females May 30th, (second brood) males August 3rd, females August 5th; and in

1932, 1st brood males and females June 3rd. The greatest number of the midges appears before 11 a.m. (standard time), the males emerging slightly before the females. The emergence of both sexes then gradually falls off, and ceases soon after 7 p.m.

A. Oviposition.

Mating is effected soon after emergence, and oviposition may take place from 3 hours to 2 days after mating. The clover flowers selected by the females are in the green-head stage (see Plate XV, A), with very little colour of the flower showing. The ovipositor is thrust down between the florets and the eggs are deposited among the hairs on the calyx singly, or in groups of from two to five. The egg is smooth and shiny, pale yellow in colour and slightly broader at one end than the other.

B. Egg stage.

The length of the egg stage varies from 2 to 6 days. In 1931, females were observed ovipositing on flower heads on June 6th. Newly hatched larvae and eggs containing segmented larvae were found on these flower heads on June 8th, other newly hatched larvae on June 10th. In 1932, ovipositing females were seen on May 30th, eggs were found on May 31st, and newly hatched larvae from June 4th to 6th. The average measurement of the eggs in 1931 was 0.290×0.056 mm., in 1932 it was 0.295×0.060 mm. This is rather smaller than the average given by Wehrle (1929) which was 0.3139×0.0849 mm. in 1921, and 0.3240×0.0790 mm. in 1922.

C. The first stage larva. ✓

Wehrle does not state the number of ecdyses passed through by *D. leguminicola*, and, indeed, Hamilton (1925) is the only investigator who has arrived at any definite conclusion regarding the number of larval instars in the Cecidomyidae. He states that *Monarthropalpus buxi* has four. Kieffer (1900) and Marchal (1897) both say that the larva passes through three phases, each of which may comprise one or more instars.

There appear to be at least four instars, distinguished from each other by definite morphological characters, in *D. leguminicola* Lint. A full account of the morphology will shortly be published.

The first instar larvae which emerge from the egg may be found on the outside of the calyx. They are small, delicate and quite transparent, measuring about 0.350×0.08 mm. Thirteen segments, including the head, are present, but the supernumerary segment or neck is not very noticeable. Antennae, mouth-parts, and eyespots are well developed. The cuticle is quite transparent and there is no sternal spatula. Only the

terminal spiracles are present and these are very prominent. The head appears much better proportioned to the body in this instar than in the subsequent ones. Entrance into the floret appears to be effected simply by eating a way through. Tiny holes in the calyx and corolla have been found which point to this method of entrance. The duration of the first instar has not been determined accurately, but cannot be more than about 2-4 days. In the case of eggs laid on May 31st, the first instar larvae were found on June 4th and the third instar on June 10th; when the eggs were laid on June 10th, the second instar larvae were found from June 14th to 16th, and the third instar larvae on June 20th, thus giving from 4 to 6 days for the second instar.

D. Second instar larva.

This is to be found within the corolla tube at the base of the ovary. It may be distinguished from the first instar larva by its greater size, the development of thoracic and abdominal spiracles, and its faintly pink colour. The cuticle is no longer smooth but cuticular papillae and spines are present though inconspicuous. The spatula is not yet present. This instar lasts from 4 to 6 days. The measurements are 1.350×0.350 mm.

E. Third instar larva.

The third instar larva is characterised by the presence of the sternal spatula or anchor process on the ventral surface of the 1st thoracic segment. The spatula is complete but is very light yellow in colour. The larva is still very pale pink and transparent, but the cuticular papillae and spines are well developed. The instar lasts about 4 days. The average measurements are 2.050×0.51 mm.

F. Fourth instar larva.

This is the full-grown larva ready for pupation and measures about 3.0×1.0 mm. It may be recognised by the well-developed, dark brown spatula and the deep pink colour of the body. In 1932, some of the larvae at this stage of development were leaving the flower heads on June 14th (eggs laid May 31st), and when placed on damp sand went down for pupation. Others remained in the heads and were still there on June 22nd, although the heads had been sprayed with water twice daily¹. Heads brought in from the same plants on June 24th contained many larvae, some of which commenced to leave the florets as soon as the heads

¹ Spraying the heads will usually cause the fully grown larvae to come out and drop to the ground. This is a useful method of finding out whether the larvae are full grown or not.

were picked. On the heads being sprayed with water, many of the larvae wriggled out. Others remained in the heads to emerge in gradually decreasing numbers every time the heads were sprayed. The last larvae dropped on July 16th.

In the summer of 1931, ten samples, each of fifty heads of clover, collected on Long Hoos Field, were brought in on June 29th and the heads were sprayed at intervals with water. The last of the larvae dropped on August 14th.

The fourth instar may therefore last as long as 7 weeks before pupation takes place. There is thus a well-marked variation in the duration of the fourth instar.

The larva leaves the head by wriggling up through the corolla tube. It then proceeds to worm itself to the edge of the head, and after hanging there a few moments, drops to the ground. This process may only take about 5 min., and there is no active springing movement. Larvae are usually much more ready to leave a flower head which has been cut, or handled, or sprayed with water.

G. Pupation.

Pupation of the first brood takes place from a few days to a fortnight after the larvae have entered the soil. These larvae spin a cocoon which is of a dull whitish colour and of a parchment-like consistency if formed as normally in the soil, or in coconut fibre, but is roughly studded with sand grains if the larvae have been placed upon sand.

In the case of the 1931 second brood, the larvae had started to drop from the flowers on August 31st, though heads containing larvae were found in the field as late as September 25th. On entering the ground the second brood larva spins a cocoon and over-winters as the fourth instar larva. During the spring of 1932, cocoons were taken from breeding pots in order to ascertain the stage of development. On May 3rd, the larvae brought in appeared to be in the pre-pupal instar; they were comparatively inactive and had assumed the stiffened attitude typical of the pre-pupa. The first pupae were obtained on May 17th and the first adults hatched on May 23rd. This gives a minimum of 6 days for pupation for this brood.

Larvae which were brought indoors and kept on damp sand at laboratory temperature developed rather earlier than those out of doors. Those brought in on April 28th had transformed into pupae by May 3rd, and the first female emerged indoors on May 10th. Others brought in on May 3rd were well-developed pupae by May 10th. By subjecting larvae

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in breeding pots to extra heat in November, Barnes found it possible to obtain adult midges in December.

The pupae are at first very pale pink in colour with opaque wings and eyes. The legs and antennae appear very much swollen. As development proceeds, the eyes gradually darken, the wings become hyaline, the legs and antennae shrink considerably and the abdomen assumes the characteristic colour of the adult. After the cocoon has been left the very delicate cuticle of the empty pupa remains behind on the surface of the soil or coconut fibre.

H. Variation in antennal segment number in the adult.

Wehrle (1924) made a study of the variation in number of antennal segments of this midge. From a study of specimens emerged under conditions of extra heat compared with those emerged under normal conditions, I have found that the proportion of individuals with the larger number of antennal segments is increased. This agrees with Barnes' conclusions (1932) when dealing with the same conditions in the case of *Dasyneura alopecuri* Reuter and *Rhabdophaga heterobia* H.Lw.

V. DAMAGE AND ATTACK.

The damage is caused by the larva which, having penetrated the calyx and corolla, feeds upon the ovary. The first point of attack appears to be at the base of the style, where a ring of brown and withered tissue may be seen. Later the larva eats out crescentic pits in the ovary. Besides the destruction of the ovary, the presence of the larva within the floret also inhibits the development of the corolla and produces a considerable thickening at the base of the corolla tube. An attacked head may be readily recognised by its uneven blooming if partially attacked, or by the suppression of the corollas if completely attacked (see Plate XV, B). It is also much harder to the touch than a normal head (see Plate XV, C).

The number of florets attacked in a head varies considerably. Some heads have been brought in with only two or three florets stunted, in other cases only this number may be normal.

In the autumn of 1929, two samples of about 100 heads each were collected at Kinsbourne Green, Harpenden, and the adults bred out the following spring. The following figures were obtained:

Sample	♂♂	♀♀	Parasites	Total	Average per head
A (100 heads)	507	646	120	1273	12.73
B (100 heads)	790	880	496	2166	21.66

This shows that the average infestation per head may vary considerably even within a small area.

In September 1931 four samples of infested heads (three of 100, one of 75) were again brought in from Kinsbourne Green. The following midges and parasites were bred from them in 1932.

Sample	♂♂	♀♀	Parasites	Total	Average per head
CLS (100 heads)	22	43	13	78	0.78
CLT (")	16	26	13	55	0.55
CLU (")	40	84	10	134	1.34
CLV (75 heads)	68	47	0	115	1.15

It will be seen that the figures are strikingly low in comparison with the figures obtained for heads from the same area two years previously. This may be due partly to the later date at which the sample was collected (September 29th (1931)—September 14th (1929)): many larvae probably had already left the heads.

A rough estimation of the attack of the spring brood on Long Hoos Field was also made in 1931. On June 29th, ten samples of fifty heads of clover, taken at random, and not selected as attacked heads as in the two previous cases from Kinsbourne Green, were brought into the laboratory. Each of these samples was kept until all the larvae appeared to have left the heads. The heads were then examined carefully for any larvae that had not dropped. Table I gives the result:

Table I.

Total number of larvae and average number of larvae per head of red clover on Long Hoos, June, 1931.

Sample (50 heads each)	Total number of larvae	Average larvae per head
1	119	2.38
2	98	1.96
3	93	1.86
4	83	1.66
5	89	1.78
6	84	1.68
7	212	4.24
8	202	4.04
9	192	3.84
10	128	2.56

Other samples of heads were taken from Long-Hoos Field on the same date, and the second brood adults and parasites bred out. Table II gives the average infestation.

Thus the infestation on Long Hoos Field in 1931 varied between 1.42 and 4.24 attacked florets per head. This can hardly be considered a serious attack, as it only works out at about 3 per cent. of the crop.

Table II.

Numbers of D. leguminicola and its parasites reared out of samples taken on Long Hoos, June, 1931.

Sample	Number of heads	♂♂	♀♀	Parasites	Total host and parasite	Average seed lost per head*
CLA	100	40	58	111	209	2.09
CLB	100	25	48	189	262	2.62
CLC	100	39	55	196	290	2.90
CLD	150	48	64	102	214	1.42
CLE	50	10	18	86	114	2.28

* Assuming each parasite is responsible for the death of one midge.

In America, records of heavy injury go back as far as 1879. Lochhead (1904) estimated that the damage done in Ontario in 1903 was one-quarter of the crop or fully half a million dollars. Jarvis (1907) stated that the loss was 25-75 per cent. Creel and Rockwood have described it as the most important pest affecting the production of clover seed.

In Great Britain, it was reported as widely destructive in the seed-growing districts in Montgomeryshire by Jenkins (1926), while Theobald (1929) described fields near Canterbury and Charing in which the crop was ruined. Other reported attacks in 1929 were as follows: in Leicester (Roebuck), in Hertfordshire (M. of A.) and in Buckinghamshire (Jary).

VI. CONTROL.

From the nature of the attack it is obviously impossible to employ insecticides as a means of control; there remain therefore natural control by parasites and cultural methods.

(a) Parasitism.

The ability of a parasite to control the outbreak of an insect pest in any particular season is a question on which there is considerable doubt and debate. Usually parasitism, to reduce the numbers of a pest to any extent, should reach 80-90 per cent.; to control a pest it should be over 90 per cent.

Various parasites, chiefly Hymenoptera, have been recorded as attacking the clover seed midge. Comstock (1880) gives *Platygaster error* Fitch and *Eurytoma funebris* Howard; Sanderson (1901) *Tetrastichus corinatus* (Forbes); Folsom (1909) two undetermined species of *Tetrastichus*; Felt (1915) *Telenomus podisi* Ashmead, *Polyrema striaticornis* Girault, *Decatoma* sp., and *Polygnotus* sp.; Wehrle (1929), *Platygaster leguminicola* Fouts and *Inostemma leguminicolae* Fouts.

Wehrle, by examining larvae found in infested clover heads, determined the percentage of parasitism to be 5.71 per cent. for *Platygaster leguminicola* and 2.86 per cent. for *Inostemma leguminicolae* in 1922.

Two species of Hymenoptera, one of which was identified by Waterston as *Tetrastichus roesellae* De Geer, were bred out from the clover-seed midge at Harpenden. For the purpose of estimating percentage parasitism the two species have been considered together.

In the 1930 spring brood from Kinsbourne Green material 616 parasites emerged and the percentage parasitism was about 16. The crest of emergence was about 1 week later than that of the host midge, being on June 6th as compared with May 30th. In the 1932 spring brood figures for parasitism were only available from four breeding pots with heads taken from the same locality. The crest of emergence was 8 days later than in the host midge, and parasitism reached 10 per cent.

In the 1931 spring brood, taken from Agdell¹, parasitism was 27 per cent. and the crest of emergence was on May 23rd, 7 days earlier than the maximum hatch of midges. Parasitism in the second brood from Long Hoos¹ was 47 per cent. and the crest of parasites emerged on July 24th, 10 days before the crest of emergence of the midges which was on August 3rd.

This alteration in relative times of emergence is interesting in view of the fact that Barnes (1930), in dealing with the relative dates of emergence of a midge (*D. alopecuri*) and its parasites on meadow foxtail grass, suggests that the variation in date of emergence of the parasite may be an important factor in increasing the magnitude of fluctuation of parasitism and so the speed at which a parasite might lose or gain its hold over its host. He also suggests that the cause of this variation may be the direct influence of climatic factors, chiefly heat and cold.

(b) Cultural methods.

(i) Variety trials and immunity.

While the clover-seed midge is most commonly found in red clover (*Trifolium pratense*), it has been reported as also occurring in white clover (*T. repens*) by MacDougall (1913), and on Alsike clover (*T. hybridum*) by MacDougall (1913), Folsom (1909) and Wehrle (1929). I have found *Dasyneura* larvae in heads of wild white clover at Harpenden, but have not succeeded in breeding the adult midges. Until this has been accomplished, no satisfactory conclusion regarding the identity of this midge can be reached.

¹ These two fields on Rothamsted Farm are widely separated, and the parasitism figures for each field are probably independent.

The variety trials were set up in order to ascertain whether any particular variety of clover is immune to attack.

Two experiments were carried out in 1931, one at Rothamsted and one at Aberystwyth.

The Rothamsted experiment. For this, the field insectary, a small outdoor house $12\frac{1}{2} \times 9\frac{1}{2}$ ft. and with two beds $12\frac{1}{2} \times 2\frac{1}{2}$ ft. was used. The insectary is shaded by a blind on the west side. The following varieties of clover, ten plants of each variety, were received from the Plant Breeding Station at Aberystwyth for the experiment:

Red clover.

1. Aa 1851. New Zealand Red.
2. Aa 1907. Wild Red.
3. Aa 1829. English Broad Red.
4. Aa 1915. English Late Red.
5. Aa 1849. Swedish Late Red.

White clover.

- I. Ac 737. Wild White.
- II. Ac 836. Dutch White.
- III. Ac 748. New Zealand White.
- IV. Ac 453. Stryno (Danish White).
- V. Ac 453. German Wild White.

Each side of the insectary was divided into five approximately equal areas, and each area into ten blocks. The clover seedlings were then randomised and planted so that each area contained one plant of each variety, red and white. The arrangement is shown in Text-fig. 1.

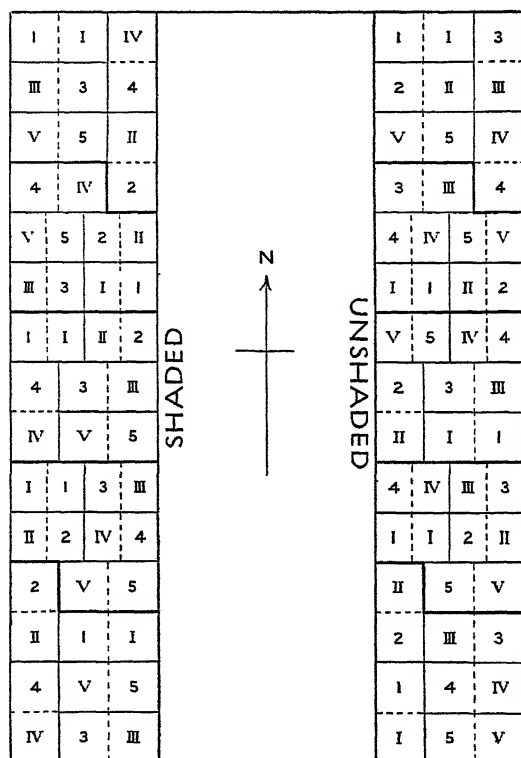
The seedlings were planted on April 23rd and were watered twice a week. All green heads were pinched off until the third week of July, when they were allowed to develop. At the beginning of August, when the second flight of midges was on the wing, there were abundant green heads on all the plants of red clover.

The insectary was infested on six successive days, August 3rd–8th, and in all 70♂♂ and 141♀♀ were liberated in the following numbers:

August 3rd	20 ♂♂	45 ♀♀
4th	4 ♂♂	18 ♀♀
5th	17 ♂♂	30 ♀♀
6th	13 ♂♂	19 ♀♀
7th	10 ♂♂	23 ♀♀
8th	6 ♂♂	6 ♀♀

After this date the insectary was not watered again until all the clover heads had been cut.

On August 25th, full-grown larvae were found in the flower heads, and appeared ready to leave the heads for pupation. On August 31st, all the heads from each plant were cut and placed in paper bags for examination.



Text-fig. 1. Plan of planting varieties of clovers in preference trial
(for explanation see text).

About midday it was observed that some of the larvae were leaving the flower heads.

In the laboratory, the heads from each plant were carefully examined, and obviously infested heads placed in glass jars and sprayed with water. The other heads were also sprayed for odd larvae. The larvae which left the heads were counted, and then placed on coconut fibre in breeding pots to serve as material for the following spring. Table III gives the results that were obtained.

Table III.
Results of the variety trial.

Variety of clover	Total heads	Infested heads	% infested	Total larvae	Average larvae per infested head
1. Aa 1851 New Zealand Red	241	101	40.8 ± 6.06	1045	8.6
2. Aa 1907 Wild Red	540	294	55.6 ± 3.66	2103	6.59
3. Aa 1829 English Broad Red	301	187	57.3 ± 4.76	1065	5.8
4. Aa 1915 English Late	351	205	58.5 ± 4.34	1648	10.7
5. Aa 1849 Swedish Late	602	458	66.4 ± 5.76	2591	5.3
I-V. Whites	138	0	0	0	0

The first point that arises is that white clover appears to be un-attacked in the presence of abundant heads of red clover. This, however, cannot be considered as proved satisfactorily. The tables show that the flower heads of white clover only averaged 2.76 per plant, whereas the red clovers averaged 40.7 flower heads per plant. This is due to some extent to the different habits of the plants, the reds developing into large and bushy plants completely overgrowing and overshadowing the white clovers. The latter have a creeping habit and their overgrowth by the red clovers appeared to inhibit flowering. For this reason, it was proposed to repeat the experiment in 1932 with the clovers alternating in groups of red and white. Unfortunately, the insectary became infested with millipedes during the winter months, and very few of the plants were unattacked. No observations are therefore available.

The second point of importance that emerges is that none of the five varieties of red clover used proved to be immune under these conditions, neither was there any suggestion that one variety is preferred to another. The percentage of attacked heads ranges from 40.8 in New Zealand to 66.4 in Swedish Late. In both cases the attack is so high as to make any apparent preference of negligible value. Again the average number of larvae per infested head is lowest in Swedish Late (5.3) and highest in English Late (10.7). Of the five varieties attacked, English Late appears to have suffered the most severely, since it combines the second highest number of heads attacked (58.5 per cent.) with the highest average number of larvae per head (10.7). The figures, however, can hardly be considered as satisfactory evidence for the preference of one clover to

another in this group. It is necessary to test other varieties of red clover.

The shading of the west side of the insectary produced no marked effects. The percentage of attacked flowers and the average number of larvae per attacked head was slightly higher on the unshaded half.

The Aberystwyth experiment. This experiment was carried out to see if there was any correlation between the time of flowering and the percentage of attack, and also to confirm the variety trials at Rothamsted. The same red clovers as for the Rothamsted experiment were used, and one white clover, viz. New Zealand White. Samples of the heads of each variety were sent from the Plant Breeding Station at Aberystwyth at fortnightly intervals during the flowering period and were received between July 2nd and October 8th. It was at first proposed that each sample should contain fifty heads of each variety, but on several occasions less were obtained as only that number of heads was in flower. The clover heads were placed in glass jars, sprayed with water at intervals, and the larvae which emerged were counted.

The following samples of heads were received:

Date	New Zealand Red	English Wild	English Broad	English Late	Swedish Late	New Zealand White
July 2nd	50	50	50	13	0	50
„ 15th	50	50	50	50	4	50
„ 29	50	50	50	50	50	50
Aug. 12th	50	50	50	50	50	50
„ 26	50	30	50	50	50	50
Sept. 9th	50	37	50	50	36	50
„ 23rd	50	37	50	50	10	50
Oct. 8th	47	10	22	34	7	50

It will be seen that full counts of heads were received from New Zealand White throughout the season. In New Zealand Red the numbers had fallen off slightly, and in English Broad rather more, in the last sample. English Wild began to fall off early in the season, while English Late which started rather later was late in falling off. Swedish Late was late in starting and early in falling off: only three times were full samples of heads received.

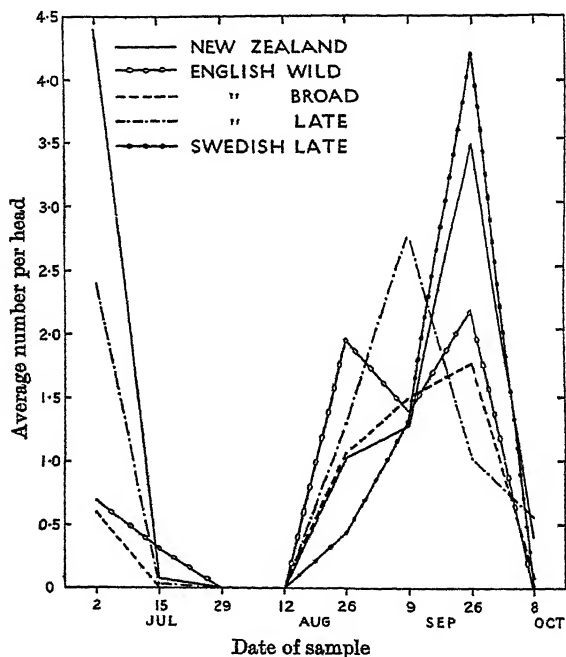
As in the Rothamsted experiment, the white clover was unattacked, while none of the red varieties was immune, all were attacked to a greater or less degree.

In Text-fig. 2 is shown the average number of larvae per head in each variety at different dates.

New Zealand Red, of which full numbers of heads were obtained on all except the last date, was most heavily attacked by the first brood with

4.4 larvae per head, and had the second heaviest attack by the second brood with 3.5 larvae per head.

English Wild, although full numbers of heads were received until August 12th, was very little attacked by the first brood (0.7). After this date the number of heads fell off and was only 10 on October 8th. The second brood attack appeared on two dates, August 26th (1.95) and September 25th (2.2).



Text-fig. 2. Infestation of varieties of clover at Aberystwyth in 1931.

English Broad shows little attack by either brood (0.6 and 1.85), English Late, although only thirteen heads were received on July 2nd, shows the second heaviest of the first brood infestation (2.4) and the third heaviest of the second brood (2.8).

Swedish Late is completely unattacked by the first brood, but although few heads were received at the end of the flowering period, was the most heavily attacked by the second brood (4.2).

It may be concluded, therefore, that of the five varieties of red clover examined, English Broad was the most satisfactorily free from clover-seed midge attack in 1931, and at the same time produced the second

largest number of heads. New Zealand, which produced the largest number of heads, suffered the worst attack. Since in the insectary trials at Rothamsted, New Zealand was not obviously preferred to English Broad by the clover-seed midge, it appears that the time of flowering must be the deciding factor. Apparently, the greatest number of the flowers of New Zealand, and the least number of the flowers of English Broad were in the green-head stage when the flight of midges was at its maximum. This suggests that if it were possible to select varieties which were at the maximum green-head stage, either some time before, or some time after the crest of emergence of the midges, the damage would be considerably lowered if not entirely absent. Unfortunately, the time of flowering as well as the date of the crest of emergence must vary with latitude, altitude and weather, and the variable nature of the English climate makes any general forecast of very doubtful reliability. It might be possible, however, to advise early or late flowering varieties for different regions and to state that these should not be in the green-head stage, say, during the week from May 28th to June 4th in Harpenden, as the crest of emergence in this locality is usually about May 30th. The unfavourable spring weather of 1932 had the effect of delaying the flowering of the clovers intended for variety trials and life history studies until well after June 3rd, when the flight of midges was at its maximum.

Immunity trials were carried out with German wild white clover, and New Zealand white, but although the females were observed ovipositing, no larvae were found and no adults reared.

(ii) *Pasturing and early cutting.*

The early cutting of the first crop of clover has been recommended by various investigators—Comstock (1880), Creel and Rockwood (1918). The object of early cutting is to catch the larvae in the flower head in the young stage, so that with the drying of the hay, the larva will be killed. Thus by the sacrifice of the first crop a second good crop of seed is ensured, since the second flight of midges will be very much reduced. Creel and Rockwood state that the heads may be left in the field until the larvae are full grown, provided there is no rain. This procedure is dangerous, because if the flowers are cut or handled in any way when the larvae are full grown, or when they are in the fourth instar, a certain number of them will leave the heads and fall to the ground. Larvae in the fourth instar even if not quite full grown are capable of pupation and will develop into normal, if rather smaller, individuals. For example, in the

1932 brood some of the larvae were ready to leave the heads a fortnight after the eggs had been laid, although the greatest numbers did not drop until they were 3 weeks old, and only then after spraying with water. *The cutting of the first crop should therefore take place as soon as possible, at least not longer than a week after the crest of emergence of the midges.* As has already been indicated this may be fixed somewhere about the end of May or the beginning of June, and farmers should be advised to cut the clover crop as soon after these dates as the attacked heads can be detected in the field. An attacked head can be detected with very little practice within a week of the eggs being laid. Co-operation between the advisory entomologist and the farmer would be of the utmost service, and it should be possible to reduce the pest to very small numbers in the course of a season.

Pasturing, and the destruction of all volunteer clovers, are also suggested as effective methods for ensuring a good second crop of seed (Creel and Rockwood, 1918). The necessity for the use of clean seed need hardly be pointed out. Various methods of killing larvae and pupae within seed have been suggested—heating the seed (Cook, 1881), the use of chloroform or carbon bisulphide (Saunders, 1882), or the use of hot water first at 120° then at 135° just before the seed is sown (Wallace, 1892).

Riley (1879) was of the opinion that if clover growing was abandoned for a few years in the infested areas, this would be an effective means of eradicating the pest.

VII. SUMMARY.

The life history of the clover-seed midge has been studied, and methods of control are discussed. The midge destroying clover seed in England is *Dasyneura leguminicola* Lint., the species common in the United States. It is typically two brooded and the second brood overwinters in the larval state. There are four larval instars.

An attempt was made to establish the immunity of a variety of red clover, but all the varieties used were susceptible to attack. White clover was not attacked. It is suggested that unless an immune variety can be produced, clovers grown for seed production should be chosen with a view to their being in the green-head either before or after the time of maximum emergence of the midges (e.g. the week from May 28th to June 4th in Harpenden). Furthermore, if cutting of the first crop is used as a means for destruction of the second brood of midges, this should take place within 10 days of the crest of emergence of the midges, the

farmer deriving what information he requires from the advisory entomologist of his particular district.

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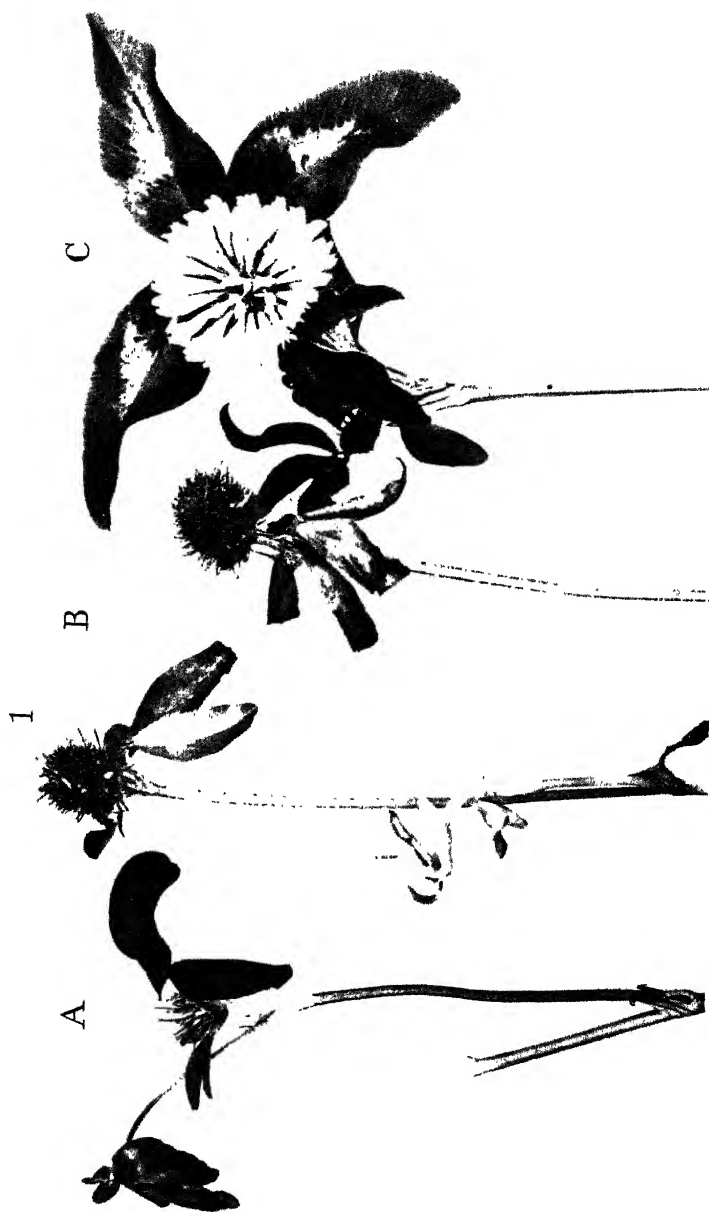
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EXPLANATION OF PLATE XV.

A, green-head in a suitable condition for oviposition; B, attacked heads with larvae (1) leaving for pupation; C, unattacked flower head of red clover.

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METCALFE.—*DASINEURA LEGUMINICOLA* (LINT.), THE CLOVER SEED MIDGE (pp. 185-204).

THE ECOTYPE CONCEPT IN RELATION TO THE REGISTRATION OF CROP PLANTS

BY J. W. GREGOR, PH.D.

(*Scottish Plant Breeding Station, Edinburgh.*)

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INTRODUCTION.

THE genetic study of the mutual relationships between plant populations and their habitats, to which the term *Genecology* has been applied by Turesson (1922), is a step towards the recognition of the smaller taxonomic units as they are realised under natural conditions. Genecological research has revealed the fact that, in nature, species-populations may contain groups of plants which, apparently by reason of the influence (primarily selective) of the prevailing environment, have come to possess a certain genotypic constitution suited to the conditions of life in their particular habitats. These aggregations of genotypes compatible with a particular habitat have been termed *ecotypes* by Turesson (1922), and their measurable characteristics are capable of numerical expression in terms of the mean or modal values of their predominant morphological and physiological features. The ecotype constitutes the smallest unit in

a new system of classification proposed by Turesson, and may be regarded as a product arising from environmental fractionation of larger genotype complexes called *ecospecies*. This latter term denotes the genotype compounds as they are present in nature, but, since these units have somewhat circumscribed capabilities which limit the possible occurrence of certain combinations under natural conditions, the term *coenospecies* has been given to designate the total sum of possible combinations in a genotype compound (Turesson, 1929).

I. THE ECOTYPE CONCEPT.

The nature of ecotypes.

The ecotype concept postulates that the ecotype unit be regarded as the result of the sorting and controlling effect of the habitat factors (*e.g.* climatic, edaphic, and biotic) upon the various genotypes within a species-population. While there is evidence that external environment can cause the origin of new genotypes, a habitat is not considered to be a directive agent responsible for the origin of genotypes specially adapted to the prevailing conditions, but, in general, as far as the genotype is concerned, is held merely to act as a passive agent, supplying an adequate environment to a population of a suitable genotypic constitution. The ecotype is, therefore, a genotypically complex unit whose constituents are capable of resisting elimination under the prevailing habitat conditions; its survival in a particular habitat is not necessarily dependent on the morphological characters phenotypically displayed, but rather on the maintenance of a state of harmony between its genotypic components and the habitat. There are, however, instances of an intimate relationship between certain definite environmental conditions and a particular habitus; in such cases the latter is sometimes the phenotypic exhibition of a special *reaction type*, which is developed by certain genotypes and is essential for their survival, *e.g.* dwarf growth in exposed situations (Turesson, 1922; Gregor and Sansome, 1927; Gregor, 1930).

Although experimental evidence indicates that an ecotype is the result of the continuous elimination of unsuitable genotypes by the environment, yet the constituent genotypes are, within limits, capable of fluctuant change as a result of the direct effect of external conditions. This modificatory capacity of individual genotypes and collective genotype groups permits within a population a range of expressional adjustment to take place in response to fluctuating environmental conditions,

and must contribute considerably to the success of such units in nature¹. While minor modifications are of frequent occurrence, it would seem that, in general, the controlling influence exerted by change of environment expresses itself more in an alteration of the proportions in which the genotypic constituents of a population occur than in a direct modificatory effect on the population as a whole. If this were not the case, the parallelism which has been demonstrated experimentally to exist between the habitats and the genotypic constitution of their populations would be much less commonly realised. It has also been observed, *e.g.* in *Succisa pratensis* Moench (Turesson, 1922) and in *Plantago maritima* L. (Gregor, 1930), that the modificatory influence of the environment may operate in a direction parallel to that of genotypic selection, *e.g.* when a population composed of hereditary dwarf variations is further reduced in height by the direct influence of its habitat conditions. Moreover, in extreme habitats, modifications may mask the normal genotypic expression to such an extent that a population, in reality composed of different genotypes, may appear to be phenotypically homogeneous. There are cases in which the degree of expressional adjustment of a genotype complex has been so pronounced that a single ecotype within one species-population has survived in two different habitats, *e.g.* *Lysimachia vulgaris* L., while in another species-population the same two kinds of habitats have been occupied by distinct ecotypes, *e.g.* *Lysimachia nummularia* L. (Turesson, 1922, p. 216).

Ecotypic adaptability.

The presence of monogenotypic ecotypes in allogamous species-populations must indeed be of rare occurrence, and consequently it should not be assumed that a population must inevitably suffer extinction when the balance between its environment and genetic constitution is disturbed. Provided that the environmental change does not result in the death of all the various genotypes, it is conceivable that a readjustment of the constituent parts will take place, until once more a state of equilibrium is attained. It is, however, to be expected that the ecotypes represented at the limits of environmental distribution will be more specialised than those occupying the central area where optimal conditions prevail. Thus the specialisation of the inhabiting forms will become more pronounced as the conditions become more extreme. The populations occupying extreme

¹ The modifications in the phenotypic expression of genotypes following environmental changes are being studied by the Division of Plant Biology, Carnegie Institution, in U.S.A. (Hall, 1932), and by the British Ecological Society in England (Marsden-Jones and Turrill, 1930).

habitats may therefore be assumed to comprise a reduced number of genotypes, and, since the power of an immobile ecotype to resist changed circumstances must depend largely on the number of its component genotypes, these highly specialised ecotypes will possess a low potential adaptability, and will, in consequence, be more liable to extermination than more richly endowed populations. Ecotypical adaptability therefore is dependent on genotypical heterogeneity.

The dispersal of ecotypes.

There is evidence that similar, though separated, habitats are frequently occupied by habitat-types which bear such a striking resemblance to each other that they may be considered as belonging to the same ecotype. This re-appearance of a particular ecotype under like environmental conditions is well illustrated by the fact that, wherever the *Hieracium umbellatum* population on the Swedish west coast has been investigated, the sea-cliff localities have been found to harbour only the broad-leaved, sea-cliff type. The beach dune localities which alternate with the cliffs have, conversely, been found to support only the dune type (Turesson, 1922, p. 337). There is a marked similarity between the populations occupying the different sea-cliffs and also between those inhabiting the various dunes within their comparatively limited area. This resemblance is probably due to the genotypes having migrated from some region in close proximity to their present habitats. On the other hand, it may happen that the invasion of similar habitats is accomplished by populations of a species-population only after these have experienced a long period of migration from their optimal centre, or centres, of dispersal across dissimilar intermediate ecological regions. In such cases the ultimate habitat populations might exhibit recognisable morphological differences as resulting from a differential elimination of genotypes during the passage of the various migrant streams.

The occurrence of spatially isolated populations (geo-ecotypes).

The establishment of ecotypes in nature presumes that impediments to free sexual reproduction, within the bounds of a species-population, are imposed by environmental conditions, and that strict spatial separation is not a necessary accompaniment of ecotypic differentiation (Gregor, 1930). On the other hand, the erection of an insurmountable barrier in the territory occupied by a genotype complex, or the transportation of a part of a genotype complex by chance agencies from one habitat, across existing obstacles to another environmentally tolerable habitat, would occasion the complete spatial isolation of the population. Therefore,

just as an ecotype is a distinct part, preserved by ecological isolation, of a larger population, so these genotypes, separated as a result of a chance circumstance, might constitute distinctive units preserved by geographical (spatial) isolation.

Numerous instances are known of the occurrence of spatially isolated, but environmentally similar, habitats harbouring different forms of the same species-population. This, however, should not for various reasons be employed as an argument against the environmental control of genotype distribution, because, firstly, a population must be more or less in tune with the environment before it can become established in any new habitat. Secondly, there is no proof that isolated populations, *e.g.* the Hawaiian snail populations which inhabit different valleys on the island of Oahu (Gulick, 1905), are not merely chance introductions of certain genotypes from an ecotype already established in a similar habitat elsewhere. Thirdly, although two habitats may originally have been populated by like genotype complexes, subsequent temporary differences in environment may have brought about a differential elimination of genes, thus leading to the occupation, by dissimilar populations, of what are now similar habitats. The actual existence of geographically isolated intra-ecotype groups, which differ morphologically from each other, has been demonstrated by Turesson (1927) within the lowland ecotype *Poa alpina pediacca*.

It would seem probable, therefore, that many of the differing populations, which are taxonomically recognisable and occur in similar, though separated, habitats, may represent parts of an ecotype, while their respective differences in character are due to a limited genotypic representation of the parent populations at the time of separation. Such differences would be maintained and accentuated as a result of spatial isolation. The term *geo-ecotype* has been proposed (Gregor, 1931) to include these units so intimately connected with the ecotype, whose characteristics, however, are dependent on geographical (spatial) isolation following their chance introduction.

Uniformity of the ecotypic units.

It has been shown that the various genotypes of a species-population, occupying an environmentally subdivided region, do not become established indiscriminately throughout the entire area, but that a grouping of suitable genotypes into more or less physiologically isolated ecotypes, takes place in accordance with the differences in habitat conditions. Individual ecotypes, therefore, do not represent the total genotype complex of the unit to which they belong. This fractionation may

either take place by the chance introduction of a few homozygous or heterozygous plants into a new habitat, or by the prohibition of certain genotypes and the entrance of others during the process of normal migration. Nevertheless, once a collection of plants has invaded a habitat, the elimination of genes within the colony may still continue; some genotypes may be immediately suppressed, some may be capable of vegetative development but fail to produce seed, others may, in this respect, be more able to cope with the habitat conditions, while still others may ripen abundant seed. It is unlikely that more than a small proportion of the seeds produced annually will reach the reproductive stage; it is, however, probable that those individuals which are capable of producing the most seed will stand the greatest chance of leaving progeny. According to Hagedoorn (1921, p. 120): "Wherever the group is continued from a fraction of the number of individuals, or where a colony is started by a few individuals, the chance of the heterozygotes to be included in the group or to have heterozygous children included, is proportionate to their frequency. Heterozygotes will produce homozygotes but not the reverse." Further, in "every case in which rare individuals having genes, not present in the majority, or in which rare individuals being impure for, or lacking in genes, common property of the majority, happen to be excluded from the number of pro-creating individuals" the population will approach more closely to the homozygous condition, *i.e.* its total *potential variability* will have been lowered. Theoretically the potential variability of a freely intra-crossing population should remain 'undiminished, but under natural conditions a reduction is made possible by the action of selective agents. Selection, therefore, constitutes the primary cause in determining the ultimate adaptive characters for which an ecotype will become stable; moreover it induces stability by intensifying the isolation of certain genotypes, thus hastening an automatic reduction of potential variability. The chance elimination of genes responsible for the expression of tolerant characters would also tend to an automatic increase in their homozygosity. The comparative uniformity, so striking a feature of many allogamous ecotypes, can, therefore, be attributed primarily to the restrictions placed by the factors of the environment on inter- and intra-ecotype crossing.

On the other hand, in strictly autogamous populations a theoretical decrease of 50 per cent. in potential variability would occur automatically in each successive generation following a chance cross. It should, therefore, be possible to find a habitat occupied by a population, which as a whole possesses potential variability but is composed of numerous distinct homozygous groups each lacking potential variability. But even

a population so constituted would be liable, when natural conditions set a limit to the number of individuals occupying a given space, to a reduction in potential variability, owing to the possible disproportionate elimination, by environmental influence and by chance, of certain genotypes, resulting ultimately in the extinction of some of the pure lines.

Influence of climatic, edaphic and biotic environmental factors.

It is well known that climatic environment determines to a considerable extent the type of vegetation in any particular region, and that, in general characteristics, the floras of geographically distant but climatically similar areas bear a resemblance to each other, although their component species may be quite distinct. Recent investigations have shown that the components of a single species-population are not distributed indiscriminately over different climatic regions, but group themselves into ecotype zones which bear a relationship to their climatic environment. This phenomenon is particularly well illustrated in cases where there is a repetition of climatic ecotype zones in widely separated areas, *e.g.* the zonation of *Armeria vulgaris* on the coasts of Norway and Greenland (Turesson, 1922).

Under natural conditions the distribution of climatic ecotypes may be either in a latitudinal, meridional (see Turesson, 1931, p. 148), or, as can be observed when passing from the lowlands to alpine situations, in a vertical direction. The different ways in which ecotype zones may be formed are, according to Sinskaja (1931, p. 58), as follows:

“(1) Continuity in zonal variability [of characters] is naturally formed in the course of the migration of the complex of types from one common focus in the direction of a gradual change of climate.

(2) Zonal localisation may arise as a result of the contact of two climatypes [climatic ecotypes] (or species) spreading from two different foci, so as to meet each other. In this latter case an intermediary zone of types may occur due to the recombination of characters. However, no such gradualness or continuity in the variability of the geographical characters can be observed here as in the first instance, and besides differences between extreme zones are also revealed in non-geographical characters.”

As an example of the first type of zonation Sinskaja cites the conclusions from her work on Eurasian forms of *Camelina*, whose migration took place, according to the available data, from one centre and proceeded in a south-east to north-west direction; and as an example of the second type of zonation she instances the discovery in East Asia of transitional stages between European and Japanese radishes.

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Peculiar habitat conditions within a climatic zone may, however, be conducive to the development of intra-zonal ecotypes, *e.g.* the edaphic differentiation of *Hieracium umbellatum* L. into one type on the shifting dunes and another distinct type, which differs from it in the extreme prostrateness of its stems, on the adjacent arenacious fields (Turesson, 1922, p. 334).

That peculiar edaphic conditions are sometimes responsible for the existence of ecotypes beyond their normal distributional range is suggested by the appearance of more southern forms on chalk, as instanced by Sinskaja (1931), and by the statement of McConnell (1919, p. 125) that the "Old Red Sandstone soils generally are said to ripen wheat furthest north of any in Scotland."

Both climatic and edaphic conditions can, however, on occasion be materially altered by the activity of plant and animal agencies, in consequence of which peculiar local environmental conditions are realised, *e.g.* those obtaining in woodland associations where specialised shade ecotypes, such as *Dactylis glomerata* var. *lobata* Drej., occur. The maintenance of pasture conditions in Britain is largely the result of biotic agents, since much of the pasture land would, if freed from the influence of herbivorous animals and rodents, revert to woodland, with the consequent creation of a new biotic sub-environment.

The examples briefly recorded above are sufficient to indicate the important part played by the individual environmental factors in the distribution of ecotypes. It is nevertheless the combined, though not necessarily equally intense, action of all the environmental influences which contributes to the success of the genotypes in a habitat.

II. THE ECOTYPE CONCEPT IN RELATION TO AGRICULTURE:

A CLASSIFICATORY SCHEME FOR CROP PLANTS.

It seems reasonable to suppose that, since the environmental conditions are in great measure responsible for the distribution and grouping of biotypes under natural conditions, the same control will be exerted upon the distribution of crop plants. But it must be borne in mind that, whereas the process operates in the wild by the continual interactions and adjustments of the plants concerned to the habitat conditions, in agriculture man can so influence the environment by cultural treatment and the elimination of competition, that he can frequently adapt the habitat to permit of the success of a particular crop. The two processes are essentially similar in that the end result is the attainment of equilibrium between the habitat conditions and the inhabiting forms. But even in agriculture there are limits to the possible extension of the range

of particular plants beyond the ecological regions prescribed by nature; yet before these limits are reached it may not always be economically advisable to endeavour to transform the environment to suit the plant, but rather to utilise plants inherently fitted for the existing environment.

The mapping and description of natural vegetation, and the study of plant associations, while they are of great general importance to the agriculturist, have so far had little direct influence on agricultural practice. The study of subspecific units in relation to environment, however, by emphasising the error of assuming a single ecological formula for each species, brings the application of ecological methods into immediate touch with the requirements of agriculture.

The agrotype.

The obvious parallelism between the processes underlying the ecological grouping of plants in the wild and the regional preferences exhibited by crop varieties suggests that some of the latter are in reality ecotypes, though many others represent smaller units distinguished by characters of an economic rather than an ecological significance. These smaller units are often more nearly equivalent to the geo-ecotype than the ecotype as defined by Turesson (1922), with the difference that, whereas the geo-ecotype arises from chance isolation of a part of a larger population, possibly an established ecotype, the agricultural variety more usually represents the result of conscious fractionation of a genotype complex accompanied by controlled isolation of the parts. This artificial selective influence which, even when applied to a natural ecotype, tends to a more or less rapid reduction in potential variability and to a consequent proportional change of type, which may or may not be parallel with previous environmental selection, affords a reason for distinguishing between the cultivated *agrotypes* and the natural ecotypes. An agrotype, moreover, is, under certain agricultural practices, more liable to extreme environmental fluctuations than are the units in the wild. In the case of a genotypically diversified agrotype of, for example, a pasture crop, the pasture is its natural (and economic) habitat; but, under pasture conditions, reproduction by seed is limited to such an extent by the grazing animal that an artificial habitat must be created for the purpose of reproduction. The environmental conditions prevailing in the latter habitat are materially different from those of the former, and may in time so influence the numerical frequency of the genotypes that successive generations will become less valuable for pasture purposes, cf. relevant paper by Levy and Saxby (1932). This distinction between the natural

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and the seed habitat is further accentuated when an agrotype, intended for a particular environment, is seeded in a climatically distinct region, e.g. the mangel crop (*Beta maritima* L.).

The assumption that, while natural selection tends to favour the type best able to propagate itself under a given environment, artificial selection strives to satisfy economic needs (cf. Kemp, 1929) has therefore an important bearing on the procedure to be employed in the preservation of a genetic constancy in crop units. This is particularly so in those heterogeneous units where (1) vegetative production rather than seed reproduction is concerned, (2) two distinct environments are necessitated by agricultural practice, or (3) where the unit is a perennial and therefore subject to seasonal environmental fluctuations. Under these circumstances an agrotype might, in course of time, become so altered as to be unrecognisable, consequently some control of stock-seed production is advisable in order to maintain the constituent genotypes at the required equilibrium. Intra-fertilising agrotypes, therefore, cannot be regarded as static under every environment, but rather as dynamic units liable to genetic change in response to alterations in the intensity of selection conditioned by the environmental factors of their habitats.

It might be argued that, since the influence of man is undoubtedly an environmental factor, his needs constitute part of the ecological conditions imposed upon a crop variety. But, since the requirements of man may lie in a direction opposed to the natural processes of environmental control, any system of crop plant classification must perforce give preference to the characters of economic importance. Allowance must also be made for the fact that popular fancy lends an economic value to many characters of no ecological worth. Units possessing differences of such a nature cannot be regarded as separate ecological units though they obviously must receive agricultural recognition, and so for these the term agrotype has already been suggested on page 213 of the present paper.

The agro-ecotype.

The ecological preferences of crop plants, however, must not be underestimated, since, even if optimal cultural conditions were to cause the economic differences between agrotypes to assume a greater agricultural significance than the differences of strictly ecological value, the reverse might be the case under more extreme conditions. In the majority of cases agrotypes could be grouped into larger units—*agro-ecotypes*—according to their ecological preferences.

The description of agrotypes.

Undoubtedly the classification of obligatory cross-fertilising crop plants presents one of the most difficult problems, because such groups might be expected to contain several genotypes exhibiting a number of different character combinations which could not, individually, be utilised for purposes of identification. Nevertheless, provided that during the seed-raising processes these intra-breeding populations are maintained under strict isolation, they may form valid agricultural units embodying the environmental preferences of their component parts. Under the prevailing strictly observational system of morphological differentiation it is extremely difficult, in many cases impossible, to provide a distinctive description, *e.g.* for some of the forage crop varieties. However, varieties which are considered to be morphologically indistinguishable from each other by customary methods of examination may possess dissimilarities of agricultural importance. These circumstances have led Kirk (1931) to advance the proposal that new varieties should be accepted for registration on the basis of economic superiority only and that the lack of a *distinctive* morphological description, or the occurrence of morphological heterogeneity, should not necessarily preclude them from registration. Such a criterion, if it were correlated with the administration of a stock-seed or habitat certification, would afford a means of dealing with agrotypes, including established local races of known ecological adaptation, which present peculiar difficulties as to their identification. This scheme, therefore, would constitute a useful step in a comprehensive system of registration.

Nevertheless, it should be pointed out that there is an urgent need for more exact methods than those commonly employed in the evaluation of morphological and physiological characters, *e.g.* length and breadth of leaf, etc., time of flowering, water requirements, etc. The utilisation of statistical methods in comparing varietal descriptions suggests a possible alternative to the profuse employment of adjectives which so frequently leads to descriptive ambiguity. Not only would statistical treatment involve the exact measurement of characters which could then be expressed in mean values and thus afford a way of comparing one heterogeneous population with another, but it would also supply data for the calculation of variability within each population. Statistical data could only be obtained from cultures of the individual components of a population, a condition which would necessitate the cultivation of a number of spaced plants representative of the population as a whole.

The grouping of agrotypes into agro-ecotypes.

In this proposed classification of crop plants the intra-fertile agrotypes, which represent the ultimate units of the system, would be grouped into ecological units or agro-ecotypes. The division of the latter units into climatic edaphic and biotic agro-ecotypes is, however, neither practicable nor desirable. These three main classes of environmental factors can, nevertheless, be utilised to indicate the combination of environmental preferences exhibited by the individual agro-ecotype, as is very briefly illustrated in the following hypothetical example.

Agro-ecotypes	<i>Herbage plants.</i> Environmental preferences			Described agrotypes
	Climatic	Edaphic	Biotic	
A	Northern, dry	Limestone	Pasture	<i>a, b, c, d</i>
B	Northern, dry	Limestone	Hay	<i>e, f, g</i>
C	Northern, moist	Peat	Pasture	<i>h, i</i>
D	Northern, moist	Peat	Hay	<i>j, k, l</i>

The administration of the scheme.

The following scheme for the administration of the proposed classification is suggested.

First, the organisation of a *State Institute* possessing facilities such as those at present obtaining at the National Institute of Agricultural Botany in England, and at the Plant Registration Station in Scotland. The duties of such an institute would be (1) the compilation and publication of *standard* descriptions of agrotypes, based on data obtained at the institute and incorporating information collected from regional trials regarding environmental preferences; (2) the grouping of agrotypes into agro-ecotypes; (3) the interchange with other state institutes of descriptions and data concerning these units; (4) the testing of units established by other state institutes, if their recorded ecological preferences suggest that an examination is advisable; and (5) the certification of stock-seed crops of registered agrotypes.

By centralising the compilation of descriptions and not entrusting it to individual workers situated in different ecological regions, the danger of describing different reaction types of the same genotype would be obviated. Promising new crop types would be submitted for examination, as at present, to the state institute by breeders within its sphere of influence, the institute being responsible for the naming and the publication of standard descriptions of the agrotypes which proved to be worthy of recognition.

Second, the co-operation of *District Institutes*, such as the existing agricultural colleges or other recognised institutes situated in different ecological regions. The purpose of these would be to observe the behaviour, under local conditions, of material supplied to them by the state institute: these data would be appended to the standard descriptions compiled by the state institute. The work of the state and district institutes should, whenever possible, be accompanied by adequate meteorological observations.

A complete survey of the agricultural crop units at present in cultivation, an investigation which under prosperous financial conditions might be desirable, is not, in the circumstances, a practical proposition. However, a definite advance in the methods of crop plant registration along the lines suggested seems possible, if attention were to be confined to varieties for future introduction, employing for comparison a limited number of the best varieties of the respective crop plants now in existence. Such a scheme, if adopted even within the limits of the British Empire, would make available to research workers and agricultural advisory officers reliable information regarding the crop plants produced in this area. If, however, a standard system of genecological nomenclature for agricultural crop varieties were to be accepted internationally, the exchange of material would be greatly facilitated. An international scheme would require the services of an *International Institute* as, for example, the existing organisation at Rome, acting as a central station for the recording and periodic publication of the data submitted to it by the state institutes situated in the various countries.

In a suggestive article on international agro-ecological research, Bensin (1930) proposes an international terminology for local variety types, but it is questionable whether his (Bensin, 1928) voluminous terminology will achieve the desired result. Such a proposal, however, has much to recommend it as an aid to the grouping of agrotypes into agro-ecotypes and as a means of facilitating the brief general summarisation of the detailed statistical descriptions of agrotypes submitted to the international institute by the state institutes.

Genecological crop units of greater magnitude.

It must be realised that within a single crop other genecological units occur where individual identities are maintained chiefly on account of their sterility relationships, or by the insufficient viability of hybrids.

Since morphological similarity is not an infallible guide to inter-fertility, e.g. the two intersterile groups, with 14 and 42 chromosomes respectively, within *Phleum pratense* L. (Gregor, 1931), the differentiation of these units cannot be adequately accomplished by the utilisation of morphological and ecological criteria alone, and therefore cytological and experimental investigation is necessary. The actual delimitation of these larger groups into *ecospecies* and *coenospecies* must, however, remain the function of the research worker, as they are beyond the prescribed scope of the state institutes.

III. SUMMARY.

(1) The ecotype concept as formulated by Turesson (1922) postulates the existence, in the wild, of sub-specific units—ecotypes—which result from the environmental fractionation of a larger population. Details are given of the occurrence of these units in nature.

(2) While the wild ecotypes are not strictly comparable with the agricultural crop units yet the general principles underlying this concept have a definite agricultural application. Suggestions are made for a standard system of crop classification which embodies data relating to the environmental preferences of its units. Two units of agricultural significance are discussed: (1) the *agrotype*, or ultimate crop unit, and (2) the *agro-ecotype*, or group of agrotypes possessing similar environmental preferences. The measures necessary for the administration of the scheme are briefly outlined.

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EVAPORATION OF WATER FROM APPLES IN RELATION TO TEMPERATURE AND ATMOSPHERIC HUMIDITY

BY W. HUGH SMITH, B.A., DIP. HORT. (CANTAB.).

*(Low Temperature Research Station, Cambridge, of the
Department of Scientific and Industrial Research.)*

(With 6 Text-figures.)

INTRODUCTION.

As far as can be ascertained from the literature of plant physiology, problems relating to the nature of water loss from isolated fleshy fruits have not up to the present received attention. But since water loss is known to play an important part in the storage of fruits, both in relation to their structure and metabolism and to the environment in which they are held, an attempt has been made to study certain aspects of evaporation from the apple and the pear.

Appleman, Kimbrough and Smith⁽¹⁾ have shown that potatoes exhibit certain internal changes tending to diminish the loss of water during the first few weeks of storage. Afterwards, evaporation assumes a steady rate until a marked rise presages the end of the storage period. It was therefore expected that similar changes would be found to occur in the apple, and experiments were devised to observe the rate of evaporation under conditions of controlled temperature and humidity continuously throughout the storage life of the fruit. This study was later extended to a critical examination of the relationship between evaporation and two of the "external" factors conditioning evaporation from the fruit, namely, temperature and humidity. A third important external factor, air movement, was as far as possible excluded from all the experiments. In addition, some minor experiments from which tentative conclusions have been drawn about the mechanism of water loss from the apple are here reported.

EXPERIMENTAL METHOD.

In any critical study of evaporation from apples by a weighing method account must be taken of the loss of weight due to respiration. Especially is this so at relatively high temperatures, when the apple is respiring rapidly. The rate of loss of weight due to respiration may, for instance,

at 15° C. be as high as 0.012 gm. per 24 hours, or even higher if the fruit is nearing the peak of respiratory activity. It is, therefore, not sufficient to weigh the fruit at intervals under controlled conditions; a reasonably accurate estimation of the amount of CO₂ evolved must be made concurrently. To this end a simple apparatus was devised whereby it was possible to obtain weight losses and respiratory data at the same time.

A glass container (6 $\frac{3}{4}$ in. height by 5 $\frac{1}{4}$ in. diameter) was fitted with a lid of plate glass, the outer edge of which was ground and vaselined to the rim, forming an air-tight seal. Over a circular hole in the centre of the lid were placed two closely fitting glass slides, with a small hole between them to allow a wire shaft, supporting the apple, to pass through. The undersides of these slides were smeared with vaseline so that they slid apart quite readily. Drops of sealing wax were then sealed on to the lid to form guides, a previous application of vaseline to the edges of the slides preventing the wax sticking and providing the necessary lubrication. The wire shaft was bent at the lower end to form a hook, to which the apple could be attached by means of a wire loop on the stem. The upper end of the wire shaft was soldered to a drawing pin, the pin of which had been removed, and a loop was made in it. When the weight of the apple bore on the shaft, the pin closed over the hole through which the wire passed, excluding diffusion of vapour or mass movement of air through the aperture.

Solutions of potassium hydroxide were used for the dual purpose of maintaining the required pressure of aqueous vapour in the atmosphere surrounding the apple and absorbing the CO₂ evolved. These solutions were introduced and withdrawn through a second hole in the lid, normally closed by a bung through which the stem of a thermometer passed. The potash solutions were prepared by weighing, and the concentrations checked by titration against standard hydrochloric acid, using phenolphthalein as an indicator. They were poured into the bottom of the container through a thistle funnel and were changed periodically throughout the experiment, sufficient time being allowed between changes to obtain a reliable reading of CO₂ by titrating the solutions.

The solutions were withdrawn from the container by siphoning into a bottle under reduced pressure, after which a further 20 c.c. of the fresh solution were introduced, shaken round the bottom of the container, and withdrawn in the same way, removing the remaining traces of the old solution. The contents of the bottle were then washed into a beaker, care being taken to rinse the tube used to deliver the solution into the bottle. The solution was then titrated by the double-titration method, using

phenolphthalein and methyl orange as indicators. The fresh solutions were also titrated, the acid equivalent of carbon dioxide for unit volume of fresh solution being subtracted from the readings of the spent solution, since it was found that the fresh solutions always contained some carbonate. A smooth respiratory curve was constructed for each apple and the values of carbon loss over corresponding periods were subtracted from the readings of loss of weight.

The containers were placed about 36 in. above floor-level in constant temperature laboratories at the Low Temperature Research Station, with a maximum temperature variation of $1/10^{\circ}$ C., and weighings were conducted *in situ*. The only disturbance necessitated in weighing was by placing the container under the balance table and drawing apart the glass slides on the lid. This permitted the wire shaft to swing freely when attached by a lead to the balance arm above. The potash solutions were changed only immediately after a weighing had been made, so as to cause the minimum of disturbance. Four or five weighings were made at each relative humidity in the later experiments and the mean corrected loss of weight per 24 hours taken as the rate of evaporation.

CHANGES IN EVAPORATION RATE WITH TIME.

In the two experiments under this heading a simpler method of weighing and of estimating respiratory losses was used, the method described above being evolved later. In these experiments the apples were removed from the containers for weighing and respiratory losses were determined on a parallel sample of apples in a desiccator by passing a stream of CO_2 -free air over the fruit and collecting the CO_2 evolved by bubbling through a Reiset tower containing a solution of potassium hydroxide. The CO_2 in the solution was subsequently determined by double titration with standard hydrochloric acid. While being less accurate than the method finally adopted, it is believed that this method is sufficiently precise to give a picture of the progress of evaporation throughout the period of storage.

In the first of these experiments three comparable samples of ten Bramley's Seedling apples were selected when about half-way through their storage life. Two samples were placed in two series of containers over solutions giving relative humidities of 75 and 95 per cent. respectively at a temperature of 3° C. The third sample was placed in a desiccator and the evolution of CO_2 was followed throughout the course of the experiment. Weighings were made at intervals of 7 days, and after each weighing the potash solution was replaced by fresh. The experiment was

continued up to the time the fruit showed signs of fungal rotting, when it was discarded.

From Fig. 1, in which the successive rates of evaporation are plotted against time, the course of evaporation throughout the period can be followed. It will be seen that, while apart from minor fluctuations there is little detectable change in the rate of evaporation at 95 per cent. R.H.¹, at 75 per cent. R.H. there is a steady and appreciable fall. The most interesting period for study had, however, been missed, and it remained to observe the changes which might take place during the early part of

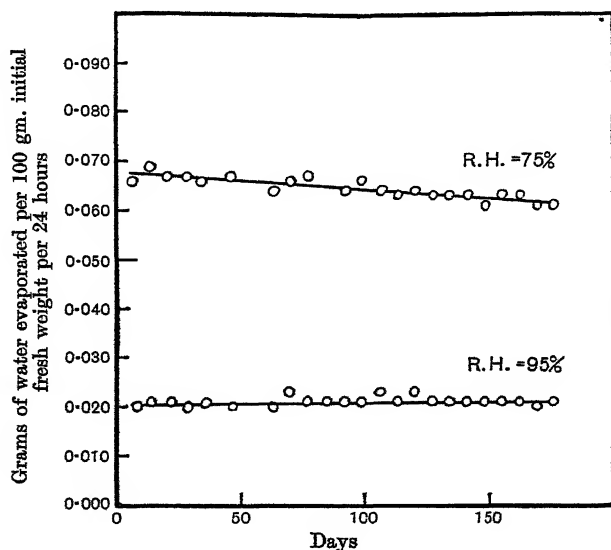


Fig. 1. Rate of evaporation of water from Bramley's Seedling apples at 3° C. and R.H. 75 % and 95 %.

the storage life when considerable modifications are known to occur in the metabolism of the fruit.

In the following year observations were made on Bramley's Seedling apples at 15 and 3° C., using 75 and 95 per cent. R.H. The apples were gathered on October 14th, 1929, and held at 3° C. before being placed in containers on October 18th. Five apples were used for each sample.

In Fig. 2 are given the rates of evaporation, corrected for respiratory losses, for the mean of each sample of apples, expressed as grams of water per 100 gm. of apple per 24 hours, from October 19th to the

¹ The abbreviation R.H. for "Relative Humidity" will be used throughout.

time the fruit was discarded owing to fungal invasion. This figure brings into striking relief the relative effects of the four different atmospheric

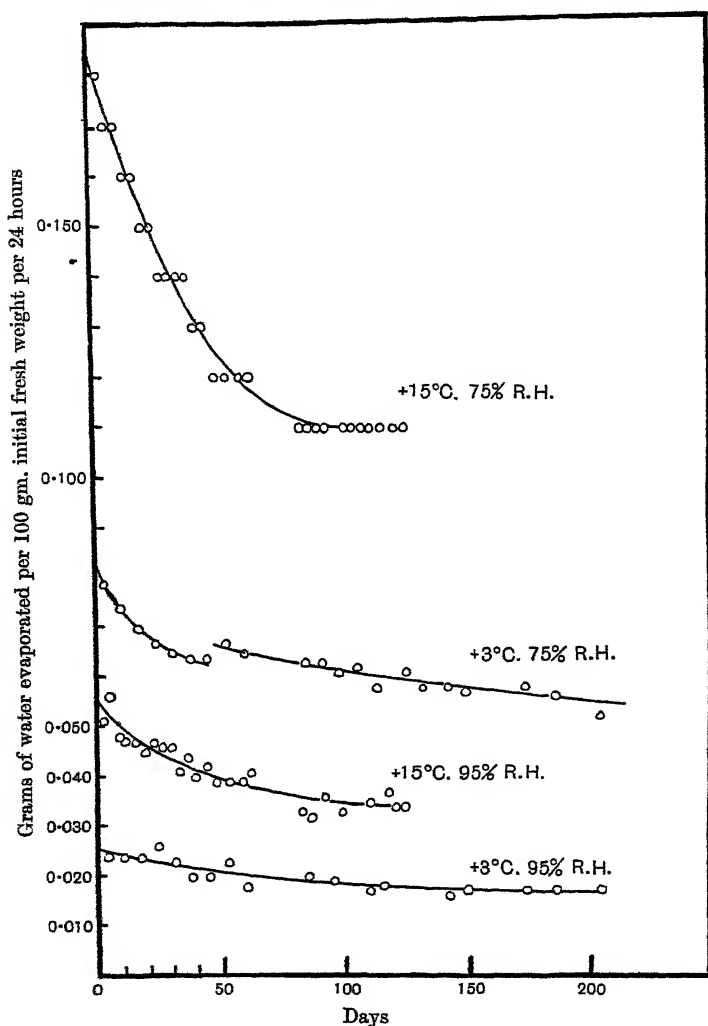


Fig. 2. Rates of evaporation of water from Bramley's Seedling apples at constant temperature and humidity.

conditions, both on the actual evaporation rate at any given time and on the changes in rate which take place during the storage period. The magnitude of evaporation rate, as would be expected, is conditioned by the vapour pressure deficit of the atmosphere, and at 15° C. and 75 per

cent. R.H. is several times greater than at 3° C. and 95 per cent. R.H. Further, the rate of evaporation during the earlier storage phase is in every case relatively high; this rate decreases rapidly at first but more slowly with increasing time. The inflection of the time-rate curve is most marked at 15° C. and 75 per cent. R.H., where a decrease in rate of 0.059 gm. per 24 hours, or 32 per cent. of the initial rate, occurs during the first 50 days. At 3° C. and 95 per cent. R.H. the inflection is slight, a decrease of only 0.005 gm. per 24 hours occurring during the first 50 days. But this is still 20 per cent. of the initial rate.

Similar changes were observed in pears of the *Beurré Clairegeau* variety, as can be seen in Table I, where the rates of evaporation of samples of the fruit at 3° C. and 75 and 95 per cent. R.H. are presented. The pears were imported at some unknown intermediate stage of life. At 75 per cent. R.H. there is a steady period, followed by a marked decline in rate up to the time the pears developed scald and rotted. At 95 per cent. R.H. the fluctuations in the readings are large, but the general tendency seems to indicate that there is no fall in rate. This relation is brought out more clearly in the fourth column, where the ratio $\frac{\text{Rate at 75 per cent. R.H.}}{\text{Rate at 95 per cent. R.H.}}$ is given for each date and a marked decline in value is evident.

Table I.

Evaporation of water from Beurré Clairegeau pears at constant temperature and humidity.

Temp. 3° C. Days	Grams of water evaporated per 100 gm. initial fresh weight per 24 hours		$\frac{\text{Rate at 75 \% R.H.}}{\text{Rate at 95 \% R.H.}}$
	95 % R.H.	75 % R.H.	
3 $\frac{1}{2}$	0.036	0.14	4.0
10 $\frac{1}{2}$	0.039	0.15	3.8
17 $\frac{1}{2}$	0.039	0.15	3.8
31 $\frac{1}{2}$	0.037	0.14	3.9
38 $\frac{1}{2}$	0.043	0.14	3.3
45 $\frac{1}{2}$	0.036	0.13	3.7
52 $\frac{1}{2}$	0.041	0.13	3.2
60 $\frac{1}{2}$	0.039	0.12	3.2

EVAPORATION IN RELATION TO RELATIVE HUMIDITY.

In this series of experiments two or three apples were used, each in a separate container as described above, and subjected successively to different atmospheric humidities, the time interval between being sufficient to allow them to reach a steady rate of evaporation. A preliminary experiment was made with Bramley's Seedling apples, selected for

uniformity of shape and size, and freedom from blemish. Attempts were made to observe the rates of evaporation in atmospheres of 65, 75, 85 and 95 per cent. R.H. at both 3 and 15° C., weighings being made at intervals

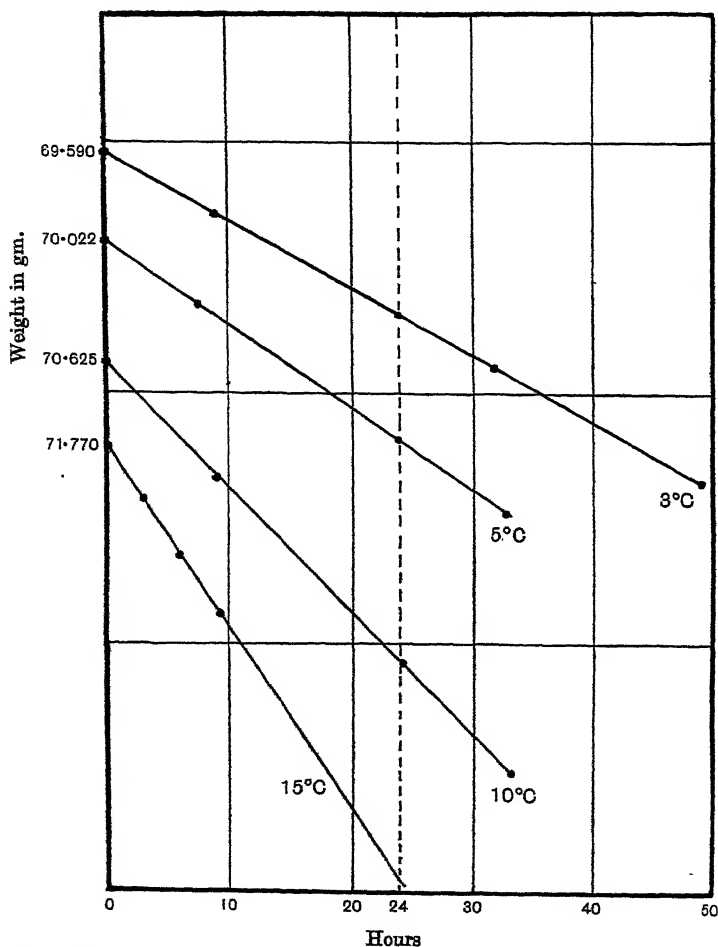


Fig. 3. Change of weight of single Cox's Orange Pippin apple (No. 9) with time.

of 2-4 days. It was found, however, that the very appreciable decrease in rate which occurs from day to day, due to internal changes in the fruit, rendered the method useless at 15° C. At 3° C. the decline in rate was slight during the period of experiment, and the curves obtained have been assumed to be accurate.

The experiment was repeated in a modified form both at 15 and at 3° C. with Cox's Orange Pippin apples. The weighings were made at the

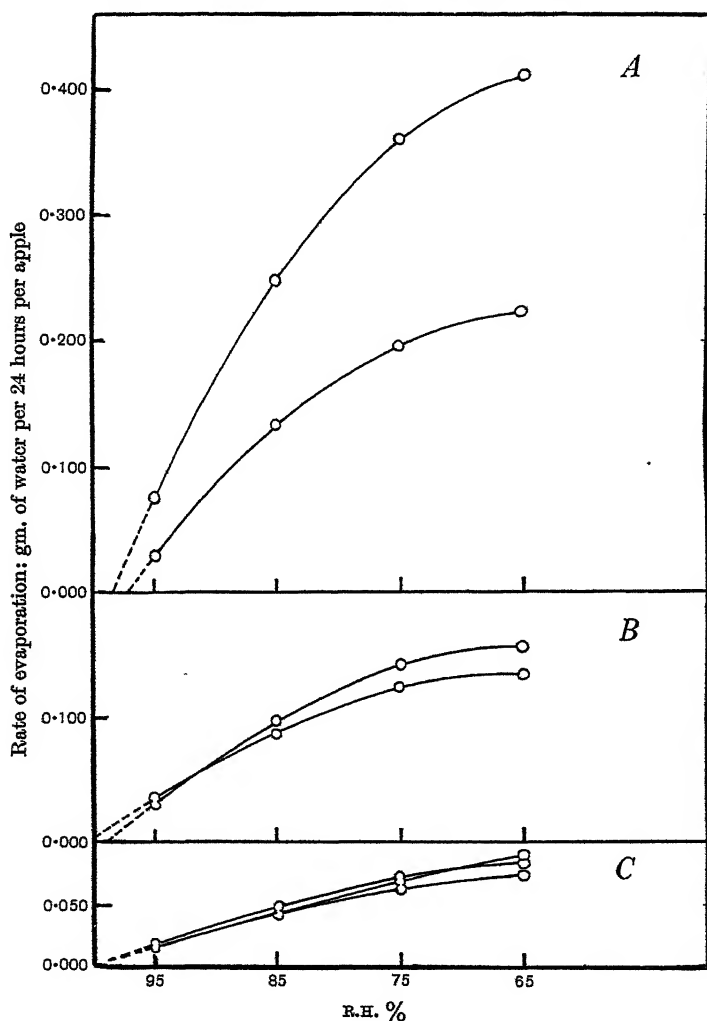


Fig. 4. Rate of evaporation from single apples at constant temperatures in relation to R.H.
A. Cox's Orange Pippin variety at +15° C. *B.* Cox's Orange Pippin variety at +3° C.
C. Bramley's Seedling variety at +3° C.

shortest possible intervals, thereby avoiding the error previously incurred. Four weighings were made per day, after which the solution was changed for one providing the next relative humidity. After an interval of

24 hours, to allow evaporation to become steady, four further weighings were made. Each set of weighings recorded was plotted against time and a straight line of closest fit drawn through the points. From Fig. 3 it will be seen that the points tend to lie very close to the line. In this way it was possible to complete the whole experiment in rather more than a week and to obtain the rate over a short period of time from the slope of the weight-time curve, thereby reducing the error due to changing rate to negligible proportions.

From the curves in Fig. 4, where the rates of evaporation from Cox's Orange Pippin apples at 3 and 15° C. and from Bramley's Seedling apples at 3° C. have been plotted against R.H., it is evident that there is no linear relationship between the two quantities, as would be expected were evaporation taking place from a free water surface. The increase in rate per unit of relative vapour-pressure deficit falls rapidly with decreasing R.H. It would, in fact, appear that below 65 per cent. R.H. decreasing R.H. would result in little increase in the rate of evaporation; possibly a steady rate might be reached with all relative humidities below 65 per cent.

It is unsafe to infer too much from curves constructed on only four points, but it is evident that the same general relationship holds at both temperatures and with both varieties of apple. Newton and Martin (4) have recorded similar phenomena in the evaporation of water from isolated fleshy segments of the cactus, *Opuntia polyacantha*. They found that the rates of evaporation were not widely different at 0, 25 and 50 per cent. R.H., but greater than at 75 or 100 per cent. R.H.

It will be noticed that the curves for the apples of both varieties at 3° C. cut the abscissa near the origin, but that at 15° C. they cut the abscissa well to the right. It would appear from this, that at 3° C. equilibrium would be established round about 100 per cent. R.H., while at 15° C. evaporation would cease at as low a humidity as 97-98 per cent. Confirmation is needed, however, before significance can be attached to this point. It is interesting to note that observations on the heat production of the apple led Smith (5) to suspect that the fruit actually absorbs water at 100 per cent. R.H.

EVAPORATION IN RELATION TO TEMPERATURE.

The method employed was similar to that used in the previous set of experiments. Two pairs of Cox's Orange Pippin apples were employed, one pair being held at 65 per cent. R.H. and the other at 95 per cent. R.H. They were placed successively at temperatures of 15, 10, 5 and 3° C., an interval of about 40 hours elapsing after removal to each new temperature

to allow the apples to reach a steady state of evaporation and respiration. The temperatures were arranged in this order so as to avoid condensation of moisture on the fruit. Four weighings were made within 24 hours and, after the last weighing, the potash solution was removed, the container transferred to the next lower temperature, and the solution replaced by another of suitable strength to give the required R.H. Under such rapidly changing conditions of temperature the readings for loss of carbon were

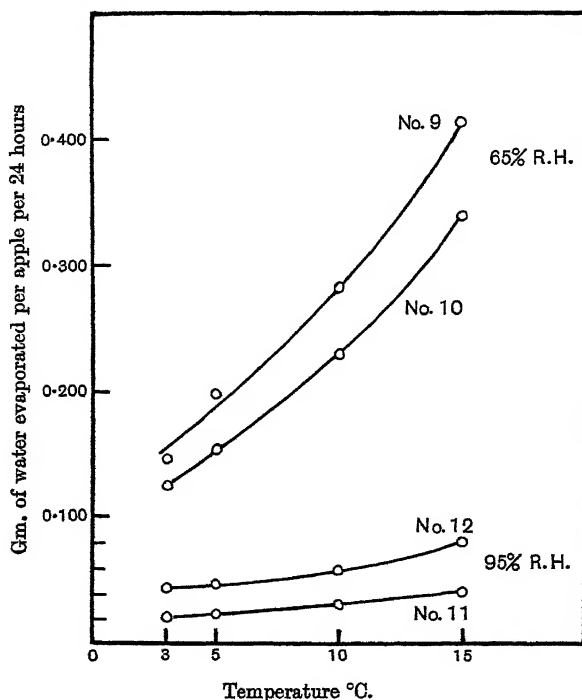


Fig. 5. Rate of evaporation of water from single Cox's Orange Pippin apples at constant R.H. in relation to temperature.

necessarily only very approximately correct, but a sufficiently good indication was obtained to allow the application of corrections to the rates of loss of weight.

The relation between evaporation rate and temperature at constant R.H. is shown in Fig. 5, where the rate of evaporation for each of the two pairs of apples at four temperatures is shown. It is evident that increase in temperature causes a considerable increase in the rate of evaporation from the apples. The curves are similar in form to those which can be

constructed to represent the relation between vapour pressure of water and temperature; a slight deviation from the vapour-pressure-temperature relationship is, however, brought out in Table II, where the values obtained for evaporation rate have been divided by the calculated vapour-pressure deficit of the atmosphere in the container. Were evaporation rate directly proportional to vapour-pressure deficit, these values should be constant. At 65 per cent. R.H., however, the values decrease with decreasing temperature; while at 95 per cent. R.H. they increase with decreasing temperature. No attempt can be made at this stage to account for this divergence.

Table II.

The relation between rate of evaporation of water from Cox's Orange Pippin apples and vapour-pressure deficit over a range of temperatures.

Temp. °C.	Vapour- pressure deficit mm. Hg	Evaporation rate Vapour-pressure deficit		Vapour- pressure deficit mm. Hg	Evaporation rate Vapour-pressure deficit	
		Apple No. 9	Apple No. 10		Apple No. 11	Apple No. 12
		gm./24 hr./mm.	gm./24 hr./mm.		gm./24 hr./mm.	gm./24 hr./mm.
		Hg	Hg		Hg	Hg
15	4.48	0.092	0.075	0.64	0.066	0.13
10	3.22	0.088	0.071	0.46	0.070	0.13
5	2.29	0.087	0.067	0.33	0.700	0.14
3	1.99	0.074	0.063	0.28	0.075	0.16

It is of interest to note the following ratios which represent the increase in rate of evaporation for a 1° C. rise of temperature, the value for a rise of temperature from 2 to 3° C. being taken as unity:

2-3° C.	4-5° C.	9-10° C.	14-15° C.	
1	: 1.1	: 1.25	: 2.1	(Apple No. 9)
1	: 1.1	: 1.3	: 2.1	(Apple No. 10)

Thus, whereas a rise of 1° C. in temperature produces at 4-5° C. only a slightly greater increase in evaporation rate than the same rise at 2-3° C., at 9-10° C. the increase in rate is one and a quarter times as great; at 14-15° C. it is more than doubled.

THE EFFECT OF FUNGAL INVASION AND DEATH.

During the course of the experiments one of the Bramley's Seedling apples developed a soft rot. Observations of water loss, respiration rate and progress of rotting were continued until the rot had spread throughout the fruit, which eventually became a mass of brown water-logged tissue. The R.H. at which the rot appeared was 65 per cent., though growth

probably started earlier at a higher humidity. The changes in the rate of evaporation of water and in respiratory activity can readily be followed in Fig. 6. During the 19 days preceding the record of appearance of the rot a steady fall in evaporation rate and a steady rate of respiration are shown. About 4 days before the rot was observed, there was an increase in the rate of respiration which became more and more rapid. The evaporation rate began to rise immediately after the rot was noted. About 11 days

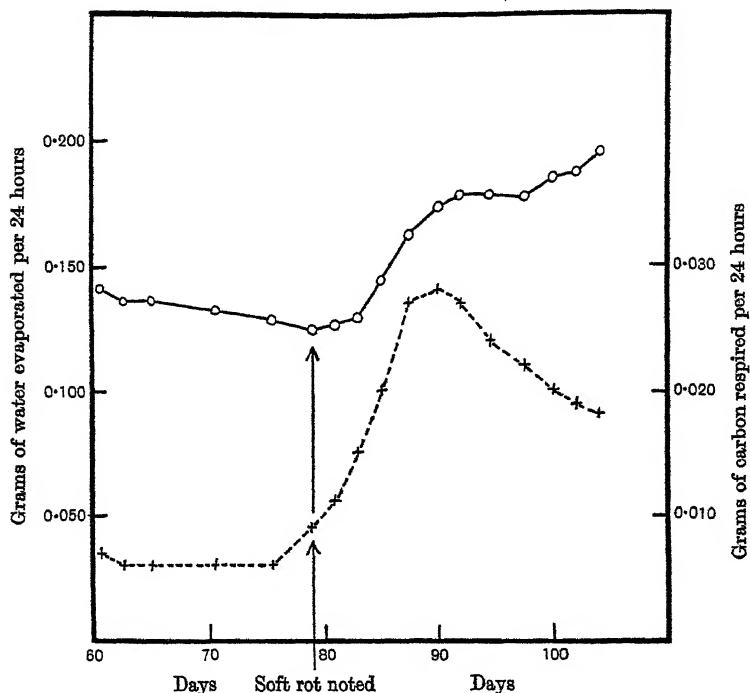


Fig. 6. Effect of rotting on rate of evaporation from Bramley's Seedling apple at 15° C. and 65 % R.H. —○—○— Evaporation rate. -- + -- + -- Respiration rate.

later respiratory activity reached a maximum, whereafter it fell rapidly and steadily. The rate of evaporation, however, continued to increase after the respiratory peak was reached, though less rapidly to the point at which the observations terminated, the apple having completely rotted.

The maximum increase in the rate of respiration over the original steady rate was 37 per cent.; the increase in evaporation rate as shown at the end of the experiment over the rate at incipient rotting being

57 per cent. This latter figure is of some interest since, if the assumption is made that death of the apple from fungal invasion results in a release of the forces tending to retain the water within the cortical tissues of the fruit, and that the fungal mycelium does not influence to any great extent the passage of water through or evaporation from the surface of the skin, it would give an indication of the relative importance of the epidermal and cortical resistances to evaporation from the fruit.

It was found that a tennis ball soaked in distilled water and then subjected to rapid centrifugal motion on the end of a string, when placed in the same apparatus as used in these experiments, gave a series of weighings which lay on a straight line. The slope of this line was taken as the rate of evaporation from a spherical free-water surface of area computed by measurement of the diameter of the ball.

The following table gives a rough comparison of the rates of evaporation per unit of surface area from: (1) sound apple, (2) completely rotted apple, (3) free-water surface. These values are used tentatively to estimate the relative importance of the epidermal and internal factors in retarding evaporation from the apple.

	Rate of evaporation of water. gm./24 hr./cm. ²
Sound tissue (epidermal and "internal" resistance)	0.0011
Rotted tissue (epidermal resistance only)	0.0017
Free water (no resistance)	0.075

It would appear that the protection afforded by the apple epidermis and the forces of resistance within the cortex is very complete, allowing only about 1/70 of the evaporation that would take place from a spherical water surface under the same conditions. In the absence of retaining forces within the cortex, the evaporation rate rises to 1/44 of the rate from the water surface; showing that the resistance provided by the epidermis is still sufficient to keep the loss of water in check.

THE APPLE CUTICLE AND EVAPORATION.

The way in which water passes from the cell vacuoles of the apple into the surrounding atmosphere is very largely a matter for conjecture. It is undecided to what extent the cuticle is permeable to water and what is the relative importance of the lenticels in the diffusion of water as vapour into the external atmosphere. Knowledge of the structure of the apple epidermis is growing rapidly. As early as 1897 Zschokke⁽⁸⁾ defined the cuticular thickening of the epidermal cells of the fruit, pointing out the existence of a wax-like layer overlaying the cuticle. Markley and

Sando(2) showed that the amount of wax deposited increases during storage. Tetley(6, 7) has shown that great variability in the structure and thickness of the deposited cuticle exists between different varieties of apples. Stomata were found to be rare in the mature apple, and lenticels were formed both from the stomata and by cracking of the epidermis at other points under strain. It has recently been shown by Markley and Sando(3) that water loss may occur from the stem and calyx ends of apples, in amounts varying from 0 to 31 per cent. according to the variety and the locality where grown.

Observations on the thickness of cuticle, very kindly made by Miss Ursula Tetley at the Low Temperature Research Station, of apples used in the evaporation experiments, are presented in Table III, together with the evaporation data. The evaporation rates are expressed per unit of surface area of the fruit. The surface area was estimated by removing the epidermis in thin rectangular strips; these were inked on the outside before drying could occur and pressed on to a sheet of bristol-board by means of a rubber roller. A clear-cut impression of each strip was obtained and its area determined by means of a planimeter. The strips were sufficiently small to avoid distortion due to curvature when lying on the board, and the sum of the areas of the impressions was taken as the surface area of the apple.

Table III.

*Rate of evaporation from Cox's Orange Pippin apples
in relation to thickness of cuticle.*

Apple No.	Mean thick- ness of cuticle Units	Mean rate of evaporation per cm. gm. water/24 hours Temp. 15° C.				
		R.H.	R.H. 95 %			
			95 %	85 %	75 %	65 %
1	232.6		0.00076	0.00253	0.00368	0.00420
2	194		0.00031	0.00150	0.00217	0.00247
R.H. 95 %						
		Temp.	3° C.	5° C.	10° C.	15° C.
12	242		0.00054	0.00058	0.00072	0.00100
11	217.3		0.00028	0.00031	0.00043	0.00057

It will be observed that there is a negative correlation between evaporation rate and mean thickness of cuticle in each of the two pairs of apples examined. It would seem therefore that other factors, such as frequency of distribution and size of lenticels and the structure of the stem and calyx ends, are of equal or greater importance in controlling the loss of water from the apple.

A note on variation.

It may be of interest to call attention to the large variations which can exist in evaporation rate between individual apples of the same variety. Considerable variation was evinced by the Bramley's Seedling apples in 1929; the maximum variations found in the four samples used being ± 20 per cent. to ± 50 per cent. of the mean. A variation of ± 50 per cent. is shown between the Cox's Orange Pippin apples Nos. 1 and 2.

SUMMARY.

A technique has been described for determining by a weighing method the rate of evaporation of water from certain isolated plant organs (apples and pears) under conditions of constant temperature and constant atmospheric humidity.

The rate of evaporation from Bramley's Seedling apples at constant temperature and humidity changes steadily throughout the storage phase; decreasing at first rapidly and then more slowly towards a more or less steady state. It is evident that the rate of evaporation is conditioned by certain internal factors, the nature of which is at present unknown; the magnitude of the control exerted by these factors is observed to change with time. No sharp changes in rate occur during this period, and so far it has not been possible to correlate the smooth transition with phenomena of metabolic activity, such as change in osmotic pressure of the cell solutions.

The relation between evaporation rate and R.H. at constant temperature shows a marked deviation from the linear, as exhibited by a free-water surface. There is an increase in the rate of evaporation with decreasing R.H. down to about 65 per cent. R.H.; a gradual decline in the rate of increase being shown for each decrement of R.H. The resistance to outward movement of water vapour would therefore appear to change with change of external vapour pressure. Below about 65 per cent. R.H. it would seem that a steady rate might be expected, when internal resistances would become limiting.

At constant R.H. and temperatures between the limits of 3 and 15° C. the rate of evaporation is more nearly approximate to the vapour-pressure deficit of the external atmosphere.

The variation in evaporation rate between individual apples of the same variety under the same conditions of temperature and humidity is considerable. These variations cannot be attributed to differences in the thickness of the cuticles, since a marked negative correlation between

evaporation rate and thickness of cuticle was found in Cox's Orange Pippin apples. Other factors such as frequency of distribution and size of lenticels and the structure of the stem and calyx ends of the fruit probably exert a controlling influence over the magnitude of evaporation rate.

The increase in rate of evaporation which occurs with fungal invasion of the tissues would seem to indicate that, although in the healthy fruit some control over the supply of water to the evaporating surface is exerted by the cortical tissues, the extent of this control is small in comparison with that exerted by the epidermis. -

Grateful acknowledgment is made to Drs Franklin Kidd and Cyril West for their very helpful criticism and advice, and to Miss Ursula Tetley for her co-operation.

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THE MOVEMENT OF TOBACCO MOSAIC VIRUS IN ITS HOST

BY J. GRAINGER, PH.D.

(*The University, Leeds.*)

(With 5 Text-figures.)

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I. INTRODUCTION.

THE experimental work to be described in this paper deals almost exclusively with the relations of a virus disease, tobacco mosaic, to the plant which it attacks. A virus has been found to increase in quantity only whilst in the tissues of the host, and it is there alone that its activities and their relations to external factors can be examined. The present paper deals mainly with the movement and distribution of virus in the host. An attempt has been made to determine the rate of spread of the virus. This involved both linear measurements on the infected host and estimations of the strength of the virus after successive intervals of time. Then the necessity for eliminating some of the forces at work within the living plant leads to the restriction of the inoculum to one part of the host separated from the rest by a barrier (*e.g.* a steamed ring). Estimations of virus spread were also performed on completely severed parts kept in humid atmospheres, and the ultimate distribution of the virus in its host was studied.

The literature dealing with previous work on the movement of a virus through its host plant has been very adequately summarised by Henderson Smith(4) and Caldwell(2).

Experimental work.

Methods. Tobacco plants of the variety Connecticut Havanna No. 132 were found most useful for general experiments, and thanks are tendered to Dr J. Johnson of the University of Wisconsin for supplies of seed. All growth before and after experimental treatment was made in an insect-free greenhouse, either at the University of Wisconsin, Madison, in 1927 and 1928, or at Weetwood, Leeds, from 1928 to 1930. The former was maintained at a temperature of 90° F., whilst the latter was considerably cooler—about 75° F. during the day. Most of the experiments were performed at Leeds, and the text indicates those which were performed at Madison. Control plants showed that not more than one seedling out of two hundred was infected by chance. Inoculation was performed by scratching the leaves with a swabbed needle which was dipped in the inoculum nine times for each plant. This resulted in the transference of 1/100 c.c. of infectious juice.

Dilution. The method of dilution of diseased plant juice has been used to test in a relative manner the distribution of a virus in different plant parts. The particular tissue was ground up in a mortar and the juice pressed out. This liquid was measured in a pipette, added to nine times its volume of water and well shaken. This was considered to be a dilution of 1 in 10. Further dilutions were made in a similar manner, a part of the 1 in 10 dilution being added to 9 parts of tap water to make a dilution of 1 in 100, and so on. The dilutions were inoculated in the ordinary way.

The method is not absolute, but only relative; dilution of an extract 1 in 10 means that the chances of the infectious agent being transferred to the plant under inoculation are reduced to one-tenth those in the extract before dilution. This element of chance is well seen in a result such as the following:

Dilutions inoculated	1/10	1/100	1/1000	1/10,000
No. of plants diseased (out of 5 inoculated)	5	4	1	0

The 1/100 dilution has only infected four plants out of the five inoculated, so it is doubtful whether the single plant which has become diseased when inoculated with the 1/1000 extract should be considered significant. It has been the arbitrary standard in the present paper to argue that an extract was infectious only if two or more plants showed disease out of the five originally inoculated.

II. THE RATE OF SPREAD AND THE RATE OF INCREASE OF A
VIRUS IN ITS HOST PLANT.*The rate of spread of the virus in the plant.*

Several batches of plants were chosen so that they were as uniform as possible, and each was then arranged in rows of five. All were then inoculated in the usual way, except that a scratch with the needle was made at right angles to the midrib. This gave a sharp limit to the area of inoculation.

One row of plants was given no subsequent treatment, being used as a control on the method of inoculation and as a comparison for the length of the incubation period. The leaves of each of the other rows were severed at a pre-determined distance from the limit of inoculation, the distance being greater the longer the time between inoculation and cut. The cut was made with a scalpel flamed between operations on each plant, and one row was treated at each time. The subsequent state of health of the plants after the leaves had been cut off was taken as an indication as to whether the virus had spread into the leaf parts proximal to the cut or no.

Spread up the leaf or stem involved the re-inoculation of a series of transverse strips 1 cm. wide. These were cut off with a sterile scalpel at the appropriate distance from the inoculation point. The corresponding strips of five plants were ground up together, and the juice used to inoculate five more healthy plants whose subsequent state of health showed whether the virus had entered this particular strip.

Similar experiments were performed on the stem. The same methods were used as for the leaf except that the large leaves were removed and inoculation was confined to a ring round the stem.

An interesting observation has been made repeatedly on these experiments on the rate of spread. It was found that in many cases the appearance of the first symptoms of disease in treated plants was delayed (as compared with an inoculated control) by the interval elapsing between inoculation and the cut. A typical case in which the disease-inoculated "controls" showed the first symptoms after 8 days may be cited. The series of plants whose leaves were severed 5 cm. away from the inoculation limit after 5 days showed the first symptoms 12 days after inoculation. Plants whose leaves were cut after 7 days did not show the first signs of disease until after 15 days. This would seem to suggest that the cutting of the leaf removes all but a very small amount of virus, which has then to begin the process of multiplication and spreading all over again, as it had from the original inoculation.

Table I.

Experimental results upon rate of spread of virus.

A. Spread down the leaf.

No. of days between inoculation and cut	No. of plants diseased when distance (in cm.) between inoculation and cut was											
	1	2	3	4	5	6	7	8	9	10	11	12
1	0	0	.	.	.	0
2	2	1	0	0	.	0
3	2	3	2	1	1	0
4	3	.	5	5	1	1
5	5	0
6	5	.	0
7	5	4	.	.	5	4	.	.	.	3	.	3

B. Spread up the leaf.

No. of days between inoculation and cut	No. of plants diseased when distance (in cm.) between inoculation and cut was							
	1	2	3	4	5	6	7	8
2	5	0
3	5	2	1
4	.	.	.	5	5	4	2	.

C. Spread down the stem.

No. of days between inoculation and cut	No. of plants diseased when five were inoculated from a strip (cm. from the inoculation point)									
	1	2	3	4	5	6	7	8	9	10
1	0
2	3	0
3	2	0	0
4	4	.	5	.	4	.
5	.	.	.	5	.	5	.	3	.	1

D. Spread up the stem.

No. of days between inoculation and cut	No. of plants diseased when five were inoculated from a strip (cm. from the inoculation point)								
	1	2	3	4	6	7	8	9	
3/4	5	0
4	4	3	1	0
5	4	4	2	0	.
7

Four plants whose growing-points were 11 cm. away from the inoculation ring, and two plants whose apices were 14 cm. away showed the first symptoms of disease (i.e. vein clearing) in the youngest leaves. Two plants where the distance was 12 cm. showed no vein clearing, but developed it the next day. Previous observation has shown that visible vein clearing appears within 24 hours of the entry of the virus into the growing region. These results therefore suggest that the distance travelled after 6 days is approximately 12-14 cm.

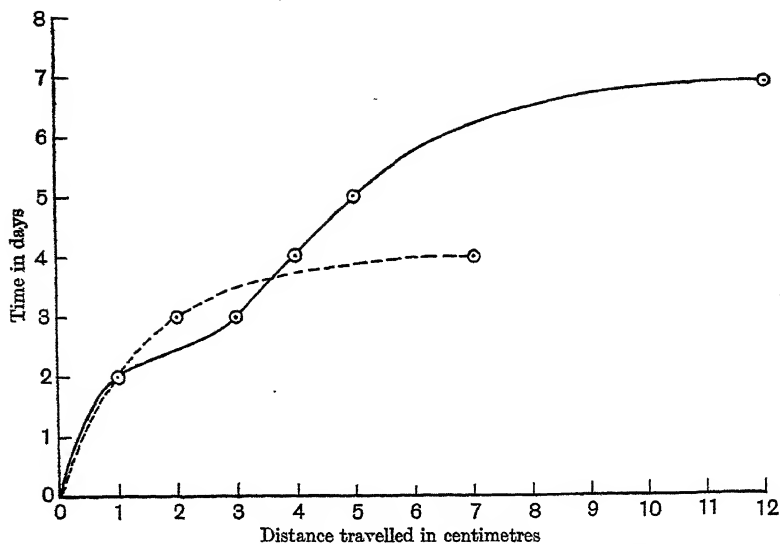


Fig. 1. The rate of movement of virus in a leaf of tobacco (*Nicotiana tabacum* L.).
 —○—○— Graph showing rate of spread down the leaf. —○--○— Graph showing rate of spread up the leaf.

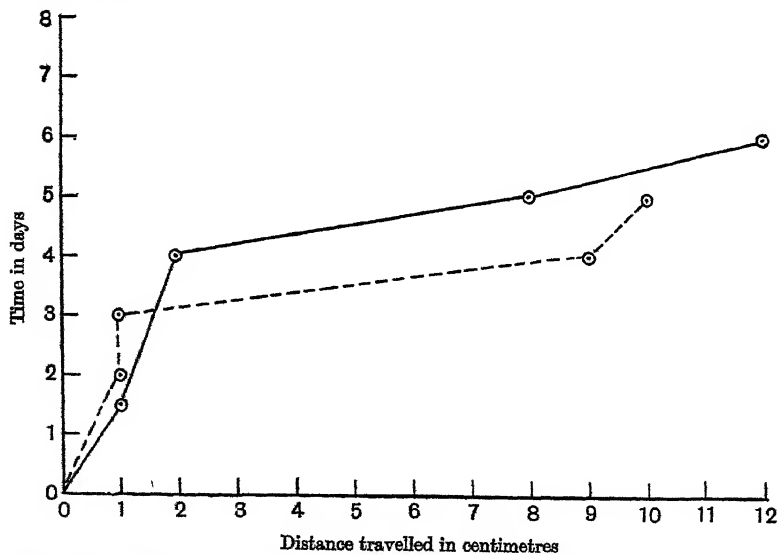


Fig. 2. The rate of movement of virus in a stem of tobacco (*Nicotiana tabacum* L.).
 —○—○— Graph showing rate of spread up the stem. —○--○— Graph showing rate of spread down the stem.

The general results suggest that the virus spreads in the plant at first slowly, but later more rapidly. When the data are expressed in the form of graphs, it is seen that there is a logarithmic relation between the distance travelled and the time taken (Figs. 1 and 2).

If the results are examined in the light of our knowledge of diffusion phenomena, it might be shown that the greatest length of spread (*i.e.* 12 cm.) *could* have been traversed in the time noted (7 days). If a very strong solution of glucose—10,000 times stronger than the limit of detection in plant tissue—had been introduced in unlimited quantity, it is possible that it could have been detected 12 cm. away from the place of introduction after 7 days. Its coefficient of diffusion would be 0.5×10^{-6} —a reasonable figure—and we should have to suppose that it was diffusing through a homogeneous plant tissue.

We do not, however, introduce unlimited quantities of the virus but only a very small amount, and there would seem to be little possibility of virus spread being caused by diffusion.

The effect of temperature on the rate of spread.

A rough indication of the effect of temperature on the rate of spread of the virus was obtained by performing in greenhouses at different temperatures comparable experiments of the type previously described. The first series was performed in the very warm greenhouses of the University of Wisconsin, Madison, Wis., U.S.A. (temperature 90–95° F.): the second trial took place in the cooler greenhouses at Weetwood, Leeds (temperature 70–80° F.). The cuts were made 3 cm. away from the limit of inoculation in both cases.

Table II.

Results.

Series I: No. of hours between inoculation and cut	18	29	30	55	70
No. of plants diseased (out of five)	0	0	2	3	5
Series II: No. of hours between inoculation and cut	48	72	96	—	—
No. of plants diseased (out of five)	0	2	5	—	—

Since light conditions were not markedly different in the two greenhouses, it seems that the rate of spread of the virus is increased by a rise in temperature.

The increase in concentration of the virus in tobacco tissue.

The leaves or stems of a large number of tobacco plants were inoculated in the usual way. At various subsequent intervals a strip or block of tissue 1 cm. wide was removed from the region next to the inoculation. The cuts were made by means of a sterile scalpel, and care was taken not

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to include any wounded tissue. The strips or blocks from five plants were ground up together, and dilutions of the juice were made and inoculated.

Table III.

Results.

A. Increase in virus concentration in the leaf.

No. of days between inoculation and treatment	Dilutions inoculated					
	1/1	1/10	1/100	1/1000	1/10,000	1/100,000
2	0	0	0	0	0	.
3	.	3	0	0	.	.
4	2	2	0	0	.	.
5	3	1	0	0	.	.
7	.	5	5	4	0	0
11	.	.	.	5	1*	0
14	.	5	5	4	1*	0

B. Increase in the stem.

No. of days between inoculation and treatment	Dilutions inoculated				
	1/1	1/10	1/100	1/1000	1/10,000
3/4	5	5	.	.	.
4	4	4	0	.	.
7	.	4	3	2	.
10	.	.	5	4	0

The figures under the dilution columns represent the number of plants which became diseased out of five inoculated with the particular strength.

* A positive result has been inferred from a single diseased plant because inoculation of the intermediate dilution of 1/5000 gave four diseased plants out of five inoculated.

The results of this experiment are perhaps more intelligible when seen in the form of graphs (Figs. 3 and 4). Part of the curve, *AB*, has the *S* shape characteristic of the rate of accumulation of the reactant in an autocatalytic reaction, a curve which is very characteristic of growth processes in which the formation of more growing substance may proportionately increase the rate of growth provided the food supplies for growth still remain unlimited (1, 6). Such a rate of increase in the virus is therefore intelligible, but throws no light at present upon the earlier curves (Figs. 1 and 2) which show a logarithmic ratio between distance and time for the spread of the virus. The most we can say at the moment is that the virus multiplies at a logarithmic rate, which suggests that of an autocatalytic chemical reaction. It is not, however, merely an autocatalytic change where a substance attacks a cell of its host and ultimately stimulates the release of more of the substance. This state of affairs would demand a linear relation between the distance travelled and the time taken. A simple consideration will show this. Suppose the virus

takes time t to reach a concentration in its host cell sufficiently high to spread a distance x to other cells. Then, when it has repeated the process of multiplication and spread, it should have taken time t and will have

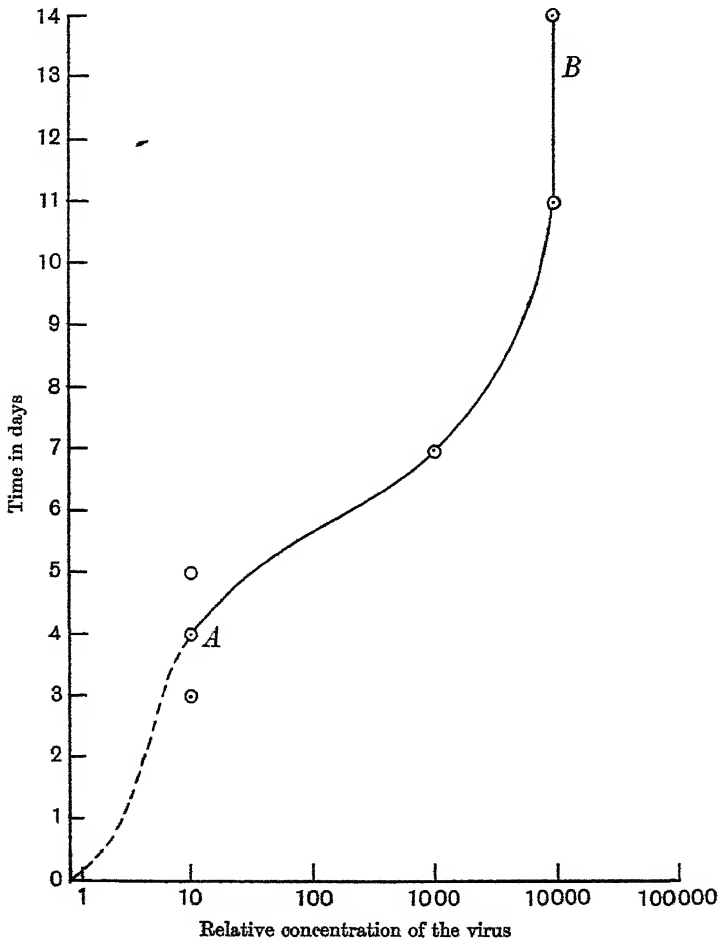


Fig. 3. Graph showing the rate of increase of a virus in a portion of a tobacco leaf.

again to spread a distance x , since the cells of the leaf do not vary greatly in diameter. So, for successive time intervals, xt will be constant, and will not increase in the logarithmic ratio which has been found to hold in practice for the virus (Figs. 1 and 2).

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III. THE SPREAD OF VIRUS IN THE SHOOT AND ROOT.

The spread of the virus to the stem and leaves.

Five tobacco plants about 4 in. high were inoculated by pricking the base of the stem immediately above the ground level. The growing-point and the three smallest leaves were removed from one plant after 4 days. They were ground up together in a mortar and the juice was used to

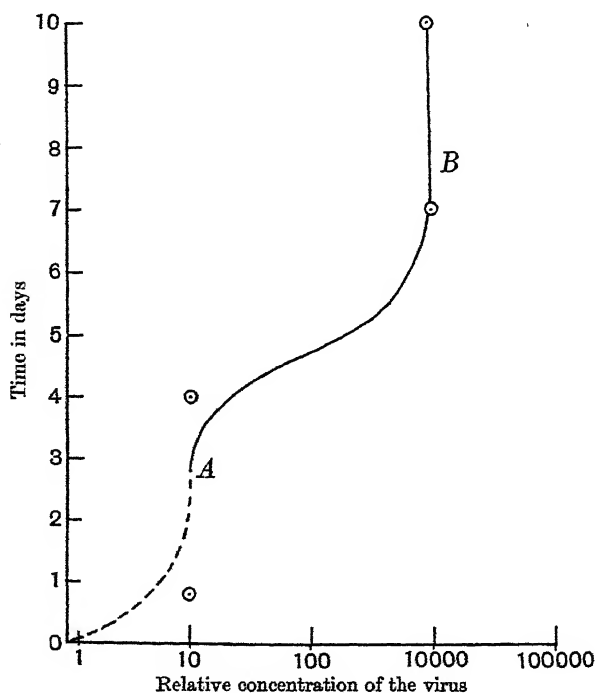


Fig. 4. Graph showing the rate of increase of a virus in a portion of a tobacco stem.

inoculate five healthy tobacco plants. The largest leaf was similarly treated at the same time and both operations were repeated after 5, 7 and 8 days, using the young leaves and an old leaf from a separate plant each time. The fifth of the originally inoculated plants had no treatment subsequent to its inoculation and was used as a control on the method.

It will be seen from Table IV that the virus passed into the youngest leaves before it entered the older ones.

Table IV.

(1) Inoculation with growing-point and top leaves.						
Days after inoculation	4	5	7	8
No. of plants diseased (out of five)	0	0	5	4		
(2) Inoculation with large bottom leaves.						
Days after inoculation	4	5	7	8
No. of plants diseased (out of five)	0	0	0	5		

A tobacco plant about 10 cm. high was inoculated by pricking at the base of the stem. Seven days later, the youngest leaf was removed, ground up in a mortar, and dilutions of its juice were prepared. The same procedure was adopted with each of the four leaves next below it.

Table V.

	Dilutions inoculated					
	1/1	1/10	1/100	1/1000	1/10,000	1/100,000
Youngest leaf	—	—	5	5	1	0
2nd expanded leaf	—	—	5	3	1	0
3rd " "	—	5	3	2	0	—
4th " "	—	1	0	0	0	—
5th " "	0	0	0	0	0	—

The figures under the dilution columns represent the number of plants which became diseased out of five healthy ones inoculated.

These results offer confirmation of the idea that the virus travels from the base of the stem first to the growing-point and young leaves. It then multiplies there and spreads to the progressively older leaves in the inverse order of their formation.

In order to study further the influence of the growing-point upon the development of the virus, four tobacco plants were inoculated at the base of the stem and the growing-point was cut out from each plant. Young leaves and old leaves were separately removed and prepared for inoculum in the usual manner. The fourth plant was left untreated as a control.

Table VI.

(1) Inoculations with young leaves.			
Time after inoculation (days)	5	7	10
No. of plants diseased (out of five)	0	0	5
(2) Inoculations with old leaves.			
Time after inoculation (days)	5	7	10
No. of plants diseased (out of five)	0	0	0

The results point to the fact that the removal of the growing-point delays the appearance of virus in the young leaves. (Cf. Table IV.)

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There were, however, 10 days after inoculation, small adventitious buds just visible. The result also offers further confirmation of the fact that the virus is recognisable first in the young leaves.

It seemed possible that the killing of stem tissue along which virus must pass might affect the ultimate distribution of the latter. In order to try out this point, several plants selected for equal size were treated by spraying a ring of the stem half way up its length with a jet of steam. They were all inoculated by pricking the stem 5 cm. below the steamed ring with swabbed needles dipped in infectious juice. After the lapse of varying intervals, the young parts above the ring and the old leaves immediately below it were separately prepared for inoculation on to healthy plants whose subsequent state of health would indicate whether the virus had passed into the corresponding parts or not.

Table VII.

Days after inoculation	6	7	8	10	14
Youngest leaves above ring	0	0	0	1	5
Old leaves below ring	0	0	5	5	—

The two bottom figures in each column represent the numbers of plants diseased out of five healthy ones inoculated.

The results show that when there is a steamed area on the stem the virus passes first to the older leaves and its entry to the younger parts is delayed.

The spread of the virus upwards and downwards across a steamed ring was tested in a similar manner. The stem was steamed and was immediately supported by tying it to a cane. The leaves were then inoculated, either above the ring or below it according to the direction in which the spread was to be tested. The part above the ring usually remained turgid for 7–10 days if the greenhouse was fairly humid, but if it was desired to test the spread of the virus upwards, the leaves and growing-point above the ring were severed after the appropriate interval and the tissue ground up, the juice being used for the inoculation of five healthy plants. If the downward spread was to be tested, the leaves above the barrier were inoculated, when the movement to the lower leaves could be judged from the state of health of the shoots which grew from the axils of the latter. Care was taken to support the upper leaves so that they did not fall on to the lower ones as they wilted and so spread the disease by contact.

It would appear from Table VIII that the virus is capable of moving upwards and downwards across a steamed part of a tobacco stem. The

steaming practically limits all effective transfer of liquids to the xylem vessels, for all other cells have their contents coagulated, and the tissue as a whole falls together.

Table VIII.

A. Spread downwards across the steamed barrier.				
No. of plants inoculated	15
No. of plants with diseased axillary shoots	...			7
B. Spread upwards across the steamed barrier.				
No. of plants inoculated	20
No. of plants whose tops produced infection when re-inoculated to healthy tobacco plants	...			10

Caldwell(2,3) has recently published the results of similar experiments, but they are in direct conflict with those here reported. The author's results seemed so definite that Caldwell's findings were somewhat of a surprise. It was, therefore, decided to repeat the experiments and, with a view to eliminating any personal equation, the writer asked Mr W. H. Douglas, B.Sc., to do one batch and Mr G. Dawson to do another. Their results are given below:

Table IX.

W. H. D. Spread downwards.				
No. of plants inoculated	10
No. of plants with diseased axillary shoots	...			5
Spread upwards.				
No. of plants inoculated	25
No. of plants whose tops produced infection when re-inoculated to healthy plants	...			4
G. D. Spread downwards.				
No. of plants inoculated	40
No. of plants with diseased axillary shoots	...			5

The passage of virus across barriers of steamed tissue on the leaves.

The leaf to be steamed was laid on a block of wood without detachment from the plant, and then two strips of moist absorbent cotton-wool were pinned to the wood so that they enclosed a narrow strip of leaf tissue between them. This part was then subjected to a spray of steam for a minute, after which the bands were removed and the part of the leaf distal to the barrier was inoculated. The subsequent state of health of the plant showed whether or not the virus travelled past the steamed area.

Table X.

Number of plants steamed and inoculated	15
Number of plants which became diseased	7

Several of these experiments were performed at Madison, Wis., U.S.A.

Investigations with red ink.

The use of red ink to trace the probable movement of the transpiration current is admittedly very crude, yet when tobacco shoots were cut and placed in a watery solution of this dye, the results were so striking when compared with the movement of the virus that they warrant a brief mention here.

When stems of tobacco were bent and cut under a solution of red ink, there was an immediate movement of the dye in some of the wood vessels, but never more than 6.5 cm. from the cut end. Presumably the wood vessels are this length in the tobacco plant. Two days later, the ink had spread into the largest leaves at the base of the stem, and was making gradual progress upwards. It had spread further after 5 days, but had not then reached the younger leaves at the apex.

A steamed ring on the stem had a marked effect, for the ink went to the young leaves first. This is in marked contrast to the virus, which seems to go to the older leaves below the ring before those at the growing-point (p. 246). These results rather suggest that the virus can move independently of the transpiration current, and it is interesting to note that Henderson Smith has, in a recent review(4), come to the conclusion that the rates of spread of most viruses are lower than the probable speed of the transpiration current.

Spread to the root.

The foregoing experiments study only the movement of the virus in the shoot, but various demonstrations of soil infection showed that the virus must be present in the root. Accordingly, four healthy tobacco plants whose stems were about 10 cm. long were defoliated with the exception of the growing-points and two expanded leaves on each. The latter and the stem between them were inoculated with ordinary tobacco mosaic virus. Two days later, a couple of the plants were carefully de-potted and the soil was washed away from the roots. Each plant was then cut up into parts, the roots, the region of inoculation including two leaves, the 3 cm. length of stem, and the tips. These were ground up separately and the strength of the juice was estimated by inoculating dilutions prepared from them. The other two plants were similarly treated after 5 days. Table XI shows the results which suggest that the virus first multiplies in the region of inoculation and travels last to the root.

Table XI.

Experiments performed at Madison, Wis., U.S.A.

I. Distribution of the virus 2 days after inoculation.

	Dilutions inoculated			
	1/10	1/100	1/1000	1/10,000
Roots	4	0	0	—
Inoculated region ...	5	5	3	3
3 cm. of leafless stem	4	0	0	0
Top of the plant ...	1	2	0	—

II. Distribution of the virus 5 days after inoculation.

	Dilutions inoculated				
	1/10	1/100	1/1000	1/10,000	1/100,000
Roots	5	5	3	—	—
Inoculated region ...	—	—	4	3	0
3 cm. of leafless stem	—	5	5	2	—
Top of the plant ...	—	5	5	2	—

The figures in the columns represent the numbers of plants diseased out of five healthy ones inoculated.

Does the virus travel in the vascular system or multiply in the lamina when inoculated at the tip of a leaf?

The leaves of fifteen tobacco plants were inoculated at the tips as described for the experiments on the rate of spread, and after 5 days eight of them had the region of inoculation cut off 2 mm. away from the inoculation limit. A strip 1 cm. wide was then cut off each of the remaining leaf portions, using a sterile knife. The midrib was then removed from each strip and the whole number of midrib pieces were ground in a mortar, the small amount of juice being used to prepare dilutions. The lamina parts were likewise ground up and estimation of the strength of juice in them obtained by the inoculation of dilutions. The midrib parts could only be regarded as being "mainly vein tissue."

Table XII.

	Midrib.	Dilution				
		1/1	1/10	1/100	1/1000	1/10,000 1/100,000
A. Severed after 5 days:						
No. of plants diseased (out of five)	3	1	0	0	0	—
Severed after 14 days:						
No. of plants diseased (out of five)	—	5	5	4	1	0
Lamina.						
Severed after 5 days:						
No. of plants diseased (out of five)	1	1	0	—	—	—
Severed after 14 days:						
No. of plants diseased (out of five)	—	5	5	2	0	0

Table XII (*contd.*).

B. Severed after 3 days.

		Dilution			
Midrib.		1/10	1/100	1/1000	1/10,000
No. of plants diseased (out of five), 1st cm.		2	1	0	0
No. of plants diseased (out of five), 2nd cm.		0	0	0	0
Lamina.					
No. of plants diseased (out of five), 1st cm.		4	0	0	0
No. of plants diseased (out of five), 2nd cm.		1	0	0	0

A variation of the experiment was made by cutting two consecutive centimetre strips 3 days after inoculation. The results are given in Table XII B.

The results of this experiment show that the virus cannot be detected more readily in the midrib tissue than in the lamina. The virus, therefore, does not only follow the veins in its spread down the leaf, but probably advances over the whole width of the lamina.

IV. MULTIPLICATION OF THE VIRUS IN DETACHED PLANT ORGANS.

The spread of the virus in detached stems.

This experiment was performed on six severed tobacco stems each about 9 in. long. Two were defoliated completely, the growing-point being also cut off. Another pair had all leaves, except two large ones at the base, removed, and again the growing-point was separated. The third pair was defoliated except that the growing-point and the two large leaves near it were allowed to remain.

Table XIII.

Series I. Leaves at bottom.

		Dilution			
		1/10	1/100	1/1000	1/10,000
No. of plants diseased (out of five):					
	Lower 3 cm.	5	3	1	1
	Upper 3 cm.	5	2	1	0

Series II. Leaves at top.

		Dilution			
		1/10	1/100	1/1000	1/10,000
No. of plants diseased (out of five):					
	Lower 3 cm.	5	1	0	0
	Upper 3 cm.	5	2	0	0

Series III. No leaves.

		Dilution			
		1/10	1/100	1/1000	1/10,000
No. of plants diseased (out of five):					
	Lower 3 cm.	5	0	0	0
	Upper 3 cm.	5	3	0	0

Each stem was inoculated near the middle of its length by pricking with a naked needle dipped in infectious juice, and all were then left on the table of a storeroom. After lying thus for 3 days, the inoculated parts were cut out and pieces 3 cm. long were taken from above and below the inoculum ring of each stem. The duplicate pieces of each series were ground up together, the resulting juice being tested for the strength of the virus by the method of dilution and inoculation (Table XIII).

The spread of virus in a detached tobacco leaf.

Ten tobacco leaves about 6-9 cm. long were cut from several plants by means of a sterile knife. Each was then inoculated at the tip as mentioned for the experiments on the rate of spread. The leaves were then arranged with their petioles dipping into moist sand so that the lamina of one leaf did not touch that of another. This was ensured by placing 4 in. unused plant labels in the sand between the leaves. Finally, a bell-jar was placed over all and the whole apparatus was transferred to the tobacco greenhouse, where it stood on the bench and was not disturbed for 7 days. After the lapse of this period the bell-jar was lifted off and dilutions were prepared and inoculated from a number of strips of lamina 3 cm. wide next to the inoculated areas and also from a number of pieces of midrib similarly treated.

Table XIV.

I. Severed after 7 days.

Midrib.					
Dilution inoculated	1/1	1/10	1/100	1/1000
No. of plants diseased (out of five)		5	5	5	2
Lamina.					
Dilution inoculated	1/1	1/10	1/100	1/1000
No. of plants diseased (out of five)		5	5	5	3

II. Severed after 7 days.

Midrib.				
Dilution inoculated	1/100	1/1000	1/10,000
No. of plants diseased (out of five)		4	0	0
Lamina.				
Dilution inoculated	1/100	1/1000	1/10,000
No. of plants diseased (out of five)		3	2	0

II A. Results of experiment performed on leaf attached to the plant for comparison.

Severed after 7 days.

Midrib.				
Dilution inoculated	1/100	1/1000	1/10,000
No. of plants diseased (out of five)		2	0	0
Lamina.				
Dilution inoculated	1/100	1/1000	1/10,000
No. of plants diseased (out of five)		1	0	0

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The leaves were somewhat flaccid after their exposure for 7 days, and the uninoculated part of the lamina had undergone a general chlorosis, some leaves being quite yellow (Table XIV).

The atmosphere under the bell-jar was completely saturated with water vapour, so transpiration was presumably non-existent. Translocation processes would, probably, also be minimal and yet under these conditions the virus moves down the leaf at about the same rate as it spreads in a leaf attached to the plant. This is a reason for regarding the spread of the virus as being potentially independent of the transpiration and translocation streams. Since this experiment was performed, Miss Purdy has published similar results.

V. THE ULTIMATE DISTRIBUTION OF THE VIRUS.

Distribution of the virus in a maturely-infected tobacco plant.

Two large tobacco plants with stems about 18 in. long and the flower buds just showing were carefully removed from their pots and the roots were well washed. The plants had been artificially infected with ordinary tobacco mosaic when they were in the seedling stage, and had developed symptoms of the disease two months before this experiment was performed.

Table XV.

I. Performed at Madison, Wis., U.S.A.

Tissue	Dilutions inoculated					
	1/10	1/100	1/1000	1/10,000	1/100,000	1/1,000,000
Leaves	—	5	5	1	4	0
Roots	—	5	5	5	0	0
Cortex and outer phloem	—	5	5	5	0	—
Xylem	5	5	5	5	2	0
Pith	5	5	5	1	0	—

II. Performed at Weetwood, Leeds.

Tissue	Dilutions inoculated					
	1/10	1/100	1/1000	1/10,000	1/100,000	1/1,000,000
Roots	—	—	2	1	0	0
Cortex and outer phloem	—	—	0	0	0	0
Xylem	—	—	5	0	0	0
Pith	—	—	0	0	0	0

The figures in the columns under the dilution values represent the numbers of plants which became diseased when five healthy tobacco plants were inoculated with the particular strength.

The stem of each plant was separated from the roots by a cut just above the ground level. All leaves were next carefully removed, the knife being frequently sterilised by flaming. Each stem was cut longitu-

dinally down the middle, when the cortex and outer phloem could be readily stripped from the xylem cylinder, which could then be lifted off the pith bit by bit. Each portion of the xylem was scraped free of any adjoining phloem and the pith was likewise cut down to exclude any internal phloem. Each tissue was then enclosed in a muslin bag and washed in a stream of running water, after which it was taken out, ground in a mortar, and the resulting juice used to prepare dilutions which were inoculated on to healthy tobacco plants. The roots and leaves were also ground up separately and used to prepare dilutions (Table XV).

The main result of these experiments is to draw attention to the high concentration of the virus in the xylem—a somewhat surprising result since the wood of tobacco consists largely of vessels.

The concentration of the virus in the light and dark green areas of a mosaic tobacco leaf.

Several leaves of tobacco diseased with ordinary mosaic were selected for the sharp delineation of their light and dark-green pattern. Each leaf was placed on a sheet of glass raised an inch above a well-illuminated sheet of white paper. This showed up the different shades in strong contrast, and they could be cut away from one another by means of a diamond-headed scalpel. The light areas were ground up together in a mortar, dilutions being prepared from the juice and used for inoculation. The dark areas were similarly treated.

Table XVI.

Results.

		Light green areas.				
Dilutions inoculated	1/1000	1/10,000	1/100,000	
No. of plants diseased (out of five),	I	I	—	4	0	
" "	"	II	5	3	0	
		Dark green areas.				
Dilutions inoculated	1/100	1/1000	1/10,000	1/100,000
No. of plants diseased (out of five),	I	I	—	—	0	0
" "	"	II	5	3	0	0

The series marked II was performed at Madison, Wis., U.S.A.

The virus is therefore present in the light green areas at a concentration ten times greater than in the dark green areas.

VI. EXPERIMENTS ON GUTTATION.

Is the water naturally guttated by a mosaic tomato plant infectious?

Nine tomato plants (var. Carter's Sunrise) were potted in large pots and were inoculated with the ordinary mosaic of tobacco. When they had shown symptoms of the disease for 30 days they were caused to guttate by the following process: The compartment of the greenhouse which housed the plants was thoroughly damped down about 8 o'clock on a November evening, and the heat supply was turned off. All plants showed abundant guttation the next morning and 2 or 3 c.c. of water were collected from the hydathodes by means of a pipette. Dilutions of this liquid were prepared, and were each inoculated on to five healthy plants which all, without exception, remained healthy, showing that naturally-induced guttation water was not infectious under the conditions of the experiment.

Experiments on guttation at high pressures.

An apparatus whose details are shown in Fig. 5 was constructed. The iron cylinder *C* and pipe *D* were coated internally with enamel so that there would be no rust contaminations. When it was desired to use the apparatus, the taps *A* and *B* were opened and water was poured into the open end of pipe *D* until it was full to the brim. A split rubber bung with a central hole was then clipped round the stem of a mosaic tomato shoot after it had been pushed through the hole of the union nut *E*. The bung was then pushed tightly into tube *D* and the nut *E* screwed down, when a good joint resulted. The tap *A* was then closed and air pumped into the apparatus till the pressure was 30 lb. per sq. in., when the hydathodes began to guttate. The drops were collected and inoculated on to healthy tobacco plants. Out of six inoculated, four became diseased. A plant inoculated with the first drops (about 5 min. after the application of pressure) became diseased.

A similar experiment was performed at Madison, Wis., U.S.A. Here the tube *D*, with the plant shoot attached, was connected directly to the water supply which was under a pressure of 90 lb. per sq. in. The mosaic tomato shoots guttated readily and the drops were infectious as soon as guttated. Fifteen healthy plants were inoculated in all, and not one of these remained healthy.

The plants used for the natural guttation and for the guttation under pressure were as similar as could be reasonably expected, and the difference in their behaviour suggests that the pressure developed during

the high-pressure experiment was sufficient to leach the living cells containing the virus.

It has been shown by Wilson(7) that naturally secreted guttation contains only traces of salts and no proteins, so that if sufficient drops could be collected from the plants guttating under pressure a test for proteins would indicate whether the contents of the cells were exuding. Half a c.c. of the liquid forced out by a pressure of 90 lb. per sq. in. was therefore collected and a drop of phosphotungstic acid was added.

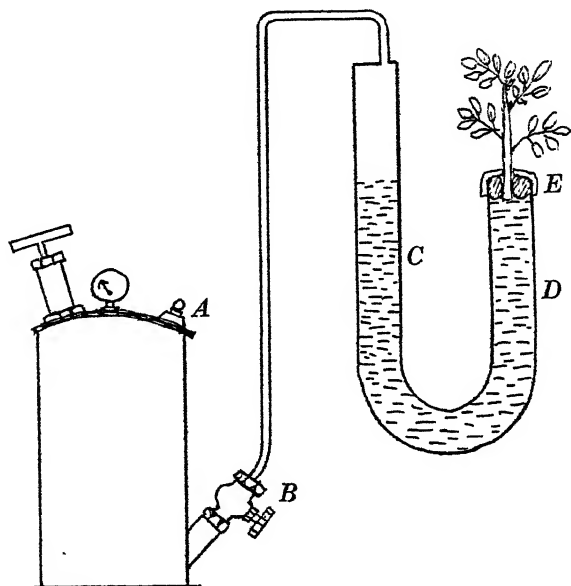


Fig. 5. Diagram of the apparatus used for guttation under pressure.

A white precipitate indicated the presence of protein-like substances. Similar results were also obtained with the guttation from healthy tomato shoots under the same pressure. We must, therefore, conclude that guttation induced by the application of high pressures is only infectious because it leaches the cells.

VII. DISCUSSION.

The experiments described in this paper show that the virus spreads through its host plant at a rate which increases logarithmically with time. Multiplication is taking place simultaneously at a different logarithmic rate which is shown by the characteristic S curve of Figs. 3

and 4. The latter curve is typical of the rate of increase of the catalyst in an autocatalytic reaction and also of many growth processes. It is, however, not so easy to find a parallel to the rate of spread. Experiments were carried out to test the ability of the virus to move in the host plant independently of any help from the transpiration or translocation streams. The results seem to show potential independence of any help from the host, in so far as the movements of food and water in the plant can be compared. Virus can make its way in the host whether the latter be a severed leaf or a growing plant. It goes to the young growing leaves before the old ones, and it can distribute itself through the whole plant after inoculation by slight pricks into the cortex of the stem. The latter fact shows that some, at least, of the movement of the virus must be through parenchyma.

What is the mechanism of this movement in the parenchyma? It has been shown on p. 241 that its rate is too quick to be caused by diffusion. Mechanical carriage by streaming protoplasm is difficult to invoke as it would seem to necessitate the postulation that cells were streaming slowly near the point of inoculation and faster further away.

It cannot be said that the virus spreads, immediately after inoculation, to a considerable distance, though not in sufficient concentration to be detected by the ordinary methods. The experiments on the rate of spread show that no plants became diseased following the severance (after 24 hours) of a leaf or stem 1 cm. away from the inoculation point. Presumably, therefore, the long distances which the virus has travelled after 5-7 days mean an increased speed of distribution of the virus, not the multiplication of previously distributed particles.

Must one look for the mechanism of spread in some function of the virus itself? The observed rate could be given by a logarithmically increasing population free to move in an area of unlimited food supply and restricted as to direction.

VIII. SUMMARY.

1. Experiments on the rate of spread of the virus of tobacco mosaic in the leaves and stems of *Nicotiana tabacum* L. show that it moves at a logarithmic rate, beginning slowly and later accelerating. The spread seems to be independent of mechanical carriage by the transpiration or translocation streams, and can certainly take place in parenchyma.

2. The virus multiplies in tissue near to the point of inoculation at a logarithmic rate portrayed by an S curve which is also typical of the

growth of living organisms and of the rate of accumulation of the catalyst in an autocatalytic reaction.

3. Naturally produced water of guttation from diseased tomato plants does not carry virus infection, but the application of high pressures ruptures the cells of the leaf and gives an infectious liquid.

The author desires to express his sincere thanks to Prof. J. H. Priestley, of Leeds University, for many helpful suggestions and advice, and to Prof. J. Johnson, of the University of Wisconsin, for help in the initial stages of the work. The work has been financed entirely by grants from the Yorkshire Cancer Research Fund.

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THE BIOLOGY OF OAT SMUTS

III. THE DEVELOPMENT OF TWO BIOLOGICAL SPECIES OF *USTILAGO KOLLERI* (WILLE) IN A SELECTION OF *AVENA STRIGOSA ORCADENSIS* (MARQUAND)

By KATHLEEN SAMPSON, M.Sc. (LOND.).

(*University College of Wales, Aberystwyth.*)

(With Plate XVI and 5 Text-figures.)

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I. INTRODUCTION.

DURING the past ten years a considerable body of evidence has accumulated to show the high degree of specialisation which exists in the species of *Ustilago* and the cereals which they attack. The oat smuts have so far received the closest study in this connection. Reed⁽¹¹⁾ has distinguished eleven biological species of *Ustilago Avenae* (Pers.) Jens., and five of *U. laevis* (K. and S.) Magn. The author⁽¹⁴⁾ has studied seven such species, one, and possibly two, of which are distinct from those described by Reed. Within the taxonomic species *sativa*, *sterilis*, *strigosa* and *brevis*, varieties and strains are found which differ within the widest limits in their reaction to particular strains of smut^(11,14).

It has been customary to describe those varieties as immune to a particular biological species which fail to develop smutted panicles when inoculated under adequate conditions. Observations recorded in recent papers provide indirect evidence that the parasite may enter the host but develop so weakly that it fails to fructify, providing thus an example of "active immunity" as recognised by Vavilov⁽¹⁶⁾. Rosenstiel⁽¹²⁾ figured and weighed plants of an immune oat variety raised from inoculated seed and showed that they were shorter and lighter in weight than plants raised from healthy smut-free seed of the same variety. A similar effect

is known in wheat, and Zade⁽²⁰⁾ considers that this "latent fungus-attack" actually justifies the fungicidal treatment of immune varieties.

Direct evidence of the invasion of resistant wheat varieties is also available since Woolman⁽¹⁹⁾ was able to trace the entrance of mycelium of *Tilletia Tritici* in such varieties as Martin, Hussar and Turkey × Florence selections. Woolman concludes from his results that highly resistant varieties can be distinguished by the slow rate of progress of the bunt mycelium in the tissues of seedlings.

The infection of oat seedlings by *Ustilago* sp. and the development of smut mycelium in the tissues of the plant were extensively studied by Brefeld⁽⁴⁾. Lang⁽⁹⁾ repeated and confirmed some of these results, and more recently Miss Kolk⁽⁸⁾, studying seedlings at all stages of growth from 1 to 40 days, has described in detail the invasion of the susceptible oat Victor by *U. Avenae*. No detailed study has been made of the growth of these fungi in the tissues of varieties which are classified as immune, although Miss Kolk states that in a preliminary examination of Black Mesdag she found mycelium in the cells of the inner epidermis of the coleoptile, but rarely anywhere else. .

Brefeld's papers contain some interesting observations on "un-successful infections." For example, in one experiment with the conidia of *U. Avenae*, which finally yielded only 20 per cent. of visibly smutted plants, Brefeld found that all the seedlings he examined had been penetrated by germ tubes of the fungus. In another similar experiment he records that a low temperature seemed to hinder the successful progress of the fungus, although it did not prevent the penetration of the seedlings by germ tubes. Brefeld came to the general conclusion that successful infection depends upon the ability of the parasite to reach and maintain a position among undifferentiated tissues before hardening sets in, and he attributed failure to the differential effect of external conditions on the relative growth of host and parasite. The possibility that the failure to infect the panicles might be due to the resistance of the host was probably not considered in this connection because Brefeld had no knowledge at that time of the existence of biological specialisation in the smut fungi. In another series of infection experiments Brefeld found that germ tubes of both maize and *Sorghum* smuts (*U. maydis* DC. and *U. cruenta* Kühn) could penetrate the tissues of young oat seedlings, but failed invariably to produce visibly infected plants. This failure was no doubt recognised as the effect of an uncongenial host.

In his recent studies of the life history of the oat smuts Gage⁽⁷⁾ discovered mycelium in the basal portion of apparently healthy plants

with normal panicles, and concluded that the failure of the fungus to attain to spore formation was due to the absence of a favourable combination of environmental factors, but he does not state if he worked with specific forms of the fungus.

Admittedly external factors have considerable influence upon infection of oats by smut fungi, but we have no definite information as to how far external influences can vary the amount of visible infection by disturbing the balance between host and parasite after infection has taken place. In order to obtain such information we need more precise knowledge of the relationship between host and pathogen when pure strains of each are brought together. Some observations relevant to this problem are described in the present paper.

Ideally the strains of host and pathogen should be genetically pure. While I am not prepared to claim so much for the two collections of *U. Kollerii*¹ under discussion, there is no doubt that they have distinct infection capacities. Their behaviour, with that of other collections on various oat varieties, was described in a previous paper (see (14), Table VI, p. 79). Additional confirmation of this behaviour on certain varieties was sought and obtained in 1931 (Table I).

Table I.

Showing the percentage smut obtained with three different biological species of U. Kollerii on three differential hosts. 1931.

Biological species	<i>A. strigosa</i> (521)	<i>A. brevis</i> (2384)	<i>A. sativa</i> potato (2855)
C 1 (Wales)	100	100	0
C 2 (U.S.A.)	100	0	100
C 4 (England)	0	0	100

The histological observations which follow relate to the behaviour of collections C 2 and C 4 on a particular selection (521) of *Avena strigosa orcadensis* (Marquand). For convenience in discussion the collections will be referred to as the virulent and non-virulent strains². The former (C 2) is capable of producing 100 per cent. visible infection on this host, while the latter (C 4) gives very low or even negative results. It is shown that under certain prescribed experimental conditions both strains of

¹ This specific name is now adopted in place of *U. laevis* used in previous publications in conformity with the list published by the Plant Pathology Sub-Committee of the British Mycological Society. *Trans. Brit. Myc. Soc.* xiv, 140 (1929).

² This distinction refers only to their behaviour on *A. strigosa orcadensis* (521). Each would be described as virulent on Grey Winter (2860) (see (14), Table VI), and on Potato (2855) (Table I, above).

smut enter the host and give rise to a considerable development of mycelium, but that whereas the virulent strain readily reaches and maintains a position not far from the growing apex, and ultimately fructifies in the inflorescence, the non-virulent strain makes slower progress, arrives later in the stele of the primary node and fails, except in rare cases, to invade the meristematic tissues and to infect the panicles. It is possible that the small percentage of visible infection given by this strain on the host under discussion is due to a slight admixture of another strain. If so it should be possible to increase the proportion of the foreign strain by selection as has been done in *T. Caries* (17). Experiments on these lines are still in progress, but any result which they may give will not materially affect the observations recorded here, since the amount of impurity, if such does exist, is obviously slight.

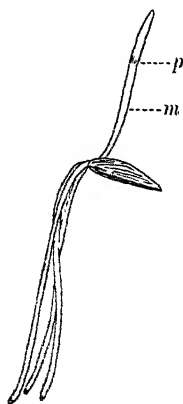
II. MATERIAL AND METHODS.

The investigation was carried out on plants grown in 1927 with the primary object of testing the resistance of certain oat varieties to different collections of smut spores. Shelled grain contaminated with the appropriate spores was germinated in sand having adequate moisture content at 22° C. (14), method C, p. 70). The final result of the test on *A. strigosa orcadensis* (521), namely, 100 per cent. with collection C 2, and less than 10 per cent. with C 3 and C 4, was confirmed by two other experiments in 1926-7, and again in 1931. The two collections C 3 and C 4 are believed to be identical, and material infected by both collections (but chiefly C 4) was used in the present study. They are jointly referred to below as the "non-virulent strain" (see p. 260).

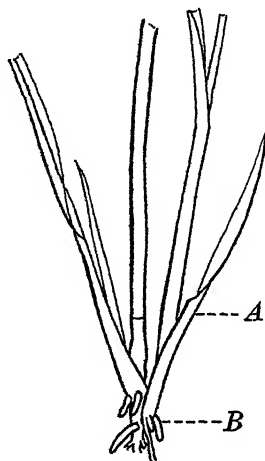
Material was fixed in 70 per cent. alcohol 5 days, 24 days, 8 weeks and 11 weeks from the date of sowing. On the first date complete seedlings were placed in the fixative, but as the plants increased in size it was necessary to cut the roots close to their base and to shorten the leaves. The primary node was always included in the part selected for fixing. The first three samples of plants were taken from pure sand, with the result that those 8 weeks old were abnormally small, but the fourth lot consisted of plants which had been transferred from sand to pots of soil at an early stage of growth. Text-fig. 2 shows a typical plant from the fourth series as it was when placed in alcohol after 11 weeks' growth.

The material was examined by two methods. Hand sections were cut and stained with lacto-phenol cotton blue, a careful record was kept of each individual plant examined, and representative slides were permanently sealed. When the main results had been established, a few

plants of each lot were embedded in wax, microtomed longitudinally 10 or 12 μ thick and stained with an aqueous solution of Bismarck brown followed by aniline gentian violet and Gram's iodine solution. These preparations completely confirmed the observations made on the hand sections, and were used for the illustrations. Transverse sections were cut to facilitate observations on the inter- or intracellular growth of mycelium.



Text-fig. 1.



Text-fig. 2.

Text-fig. 1. Typical plant of *A. strigosa orcadensis*, 5 days old. *p*, primary node; *m*, mesocotyl. Natural size.

Text-fig. 2. Typical plant of *A. strigosa orcadensis*, 11 weeks old. *A*, Position of the primordia of the inflorescence in the main tiller. This is the highest point reached at this stage by mycelium of C 2, the virulent strain of smut. *B*, Primary node. Mycelium of C 4, the non-virulent strain of smut, occurs at this level and below. $\frac{2}{3}$ natural size.

The diagrams (Text-figs. 3 and 4) aim at presenting the relative extent of the distribution of mycelium in the two series of plants under discussion. They were prepared by selecting a nearly median section and drawing it in outline under the camera lucida. Pieces of mycelium in this and in several neighbouring sections, which were deeply stained and therefore visible under a low power lens, were then added to the outline, the same apparatus being used. Had it been practicable to use a higher magnification some additional mycelium which was stained more faintly could have been included, but this would not have affected materially the area of invasion. Certain pieces of mycelium more highly magnified are shown in Plate XVI and in Text-fig. 5.

III. PRESENTATION OF RESULTS.

The number of plants examined and the presence or absence of mycelium are shown in Table II.

Table II.

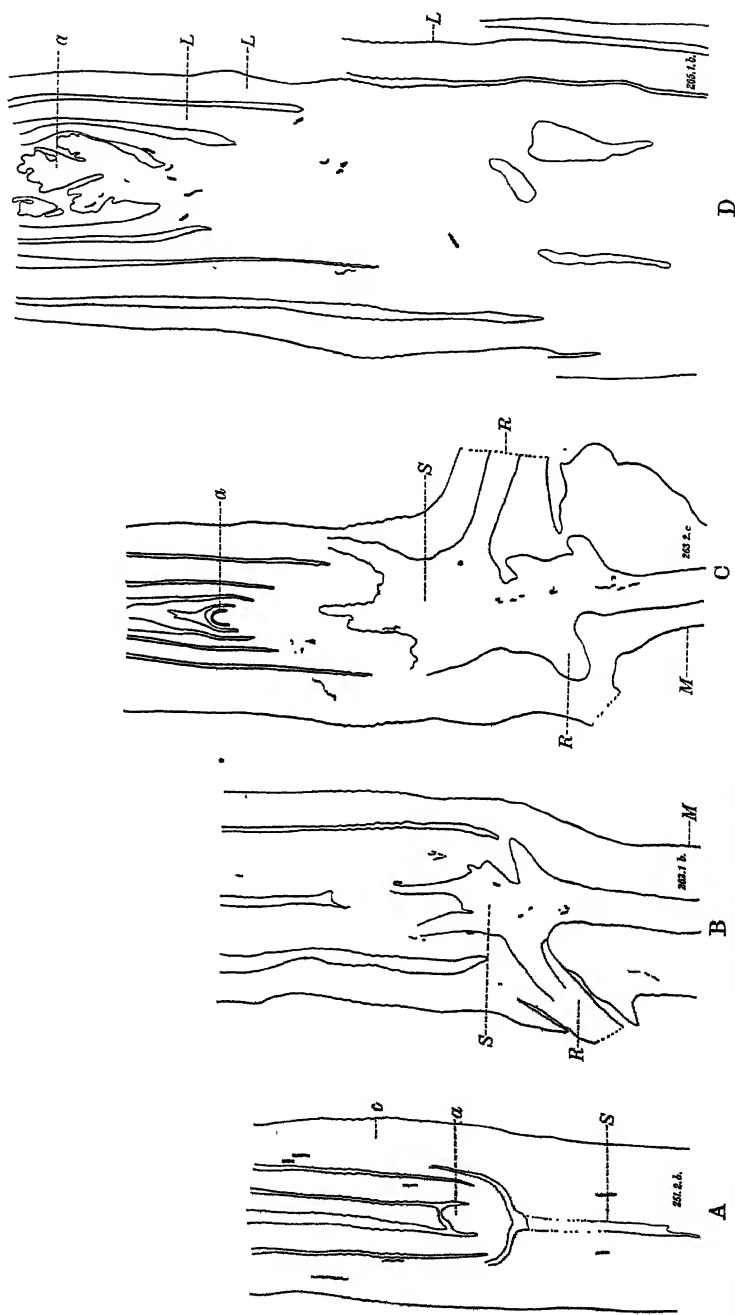
Showing the number of plants examined at each stage of development, and the number (in brackets) of plants in which the fungus was found.

	Days after sowing. Season 1927				Percentage plants with smutted panicles	
	5	24*	56	77	1927	1926-31
A. Virulent strain (C 2 on <i>A. strigosa</i> 521)	5 (5)	5 (5)	10 (10)	5 (3)	100	100
B. Non-virulent strain (C 3 or C 4 on <i>A. strigosa</i> 521)	5 (5)	5 (5)	10 (10)	5 (2)	2.5	2.3

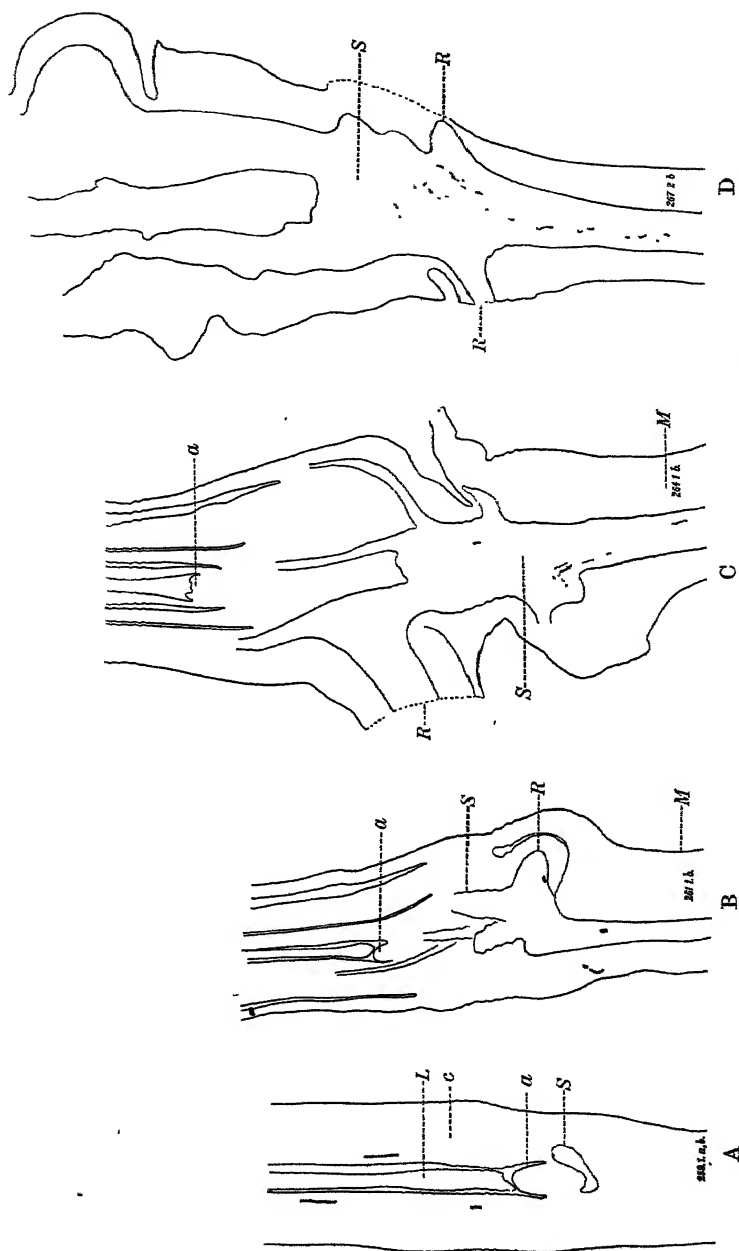
* The figures given in this column include two seedlings of each series examined after 18 days.

The first three samples exhibited remarkable uniformity among individual plants. The larger specimens, fixed after 11 weeks' growth, were less consistent, and unfortunately the number of plants available was limited. In view of the complete infection produced by the virulent strain in the mature plant, it is somewhat surprising that two plants in Series A appeared to be free from infection upon microscopic examination. It is less surprising that mycelium was recorded at this stage in only two of the five plants in Series B, since only a small part of the mesocotyl was available for examination and this proved to be particularly hard and difficult to section. The plant chosen for illustration (Text-fig. 4 D) represents that with the greatest amount of mycelium present at this stage of growth. With this exception individual plants call for no special comment and the results are presented in a generalised form.

A. *The virulent strain* (C 2). The stage of development reached by a seedling 5 days old under the conditions of this experiment is illustrated in Text-fig. 1. The elongation of the mesocotyl has carried the primary node to a distance of more than a centimetre from the point of origin of the primary roots. A certain amount of stelar tissue has already been formed, but there is little or no lignification of the cells which are destined later to form a sheath of sclerenchyma round the stele. Mycelial strands of the smut are abundant in the cortex of the mesocotyl passing from cell to cell in radial and tangential directions. They also occur in the hypocotyl and first leaf (Text-fig. 3 A).

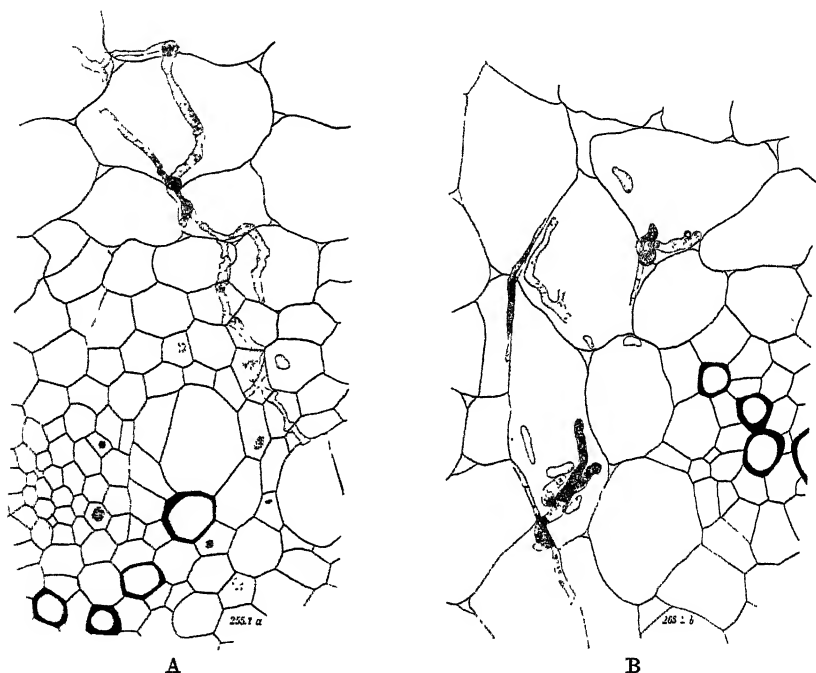


Text-fig. 3. Seedlings of *A. strigosa* (521) infected by a virulent strain (C2) of *U. Kollerii*. The sections, which are approximately median, have been drawn in outline under the camera lucida; magnification $\times 26$. All the mycelium visible without high magnification in several sections has been added to the diagram in order to illustrate the distribution of the parasite at each stage of development figured. A = 5 days; B = 24 days; C = 8 weeks in pure sand, primary node and growing point of main tiller; D = 11 weeks in soil, apex of main tiller. a, apex of shoot; M, mesocotyl; R, root; S, stela of stem; c, coleoptile; L, leaf-base. Mycelium is indicated by red lines.



Text-fig. 4. Seedlings of *A. strigosa* (521) infected by non-virulent strain (C 3 and C 4) of *U. Kollerii*. Magnification $\times 26$. A=5 days; B=24 days; C=8 weeks in pure sand, primary node and growing-point of main tiller; D=11 weeks in soil. Base of primary node, *a*, apex of shoot; *M*, mesocotyl; *R*, root; *S*, stiele of stem; *c*, coleoptile; *L*, leaf-base. Mycelium is indicated by red lines.

After 24 days roots are found emerging from the primary node, which now possesses a well-developed stele with a considerable amount of lignified tissue. Mycelium of the parasite is found readily in the stele following the parenchyma. It is also abundant in undifferentiated tissue not far from the growing-point, and it can be found occasionally in the young leaves (Text-fig. 3 B).



Text-fig. 5. A, Intracellular mycelium passing from cortex to stele in the mesocotyl of a seedling 5 days old cut transversely. The sclerenchymatous sheath is not yet formed. $\times 380$. B, Intercellular and intracellular mycelium in the axis of a lateral tiller cut transversely. A. *strigosa*, 11 weeks old, infected with the virulent strain of smut (C 2). $\times 380$.

As explained previously the 8 weeks old plants were grown in sand and were in consequence relatively stunted in growth. They differed from the previous set only in the more extensive development of the primary node and in the greater number of leaves. The distribution of mycelium in the stele and above it also corresponds closely with that described above (Text-fig. 3 C).

A typical plant taken from soil at 11 weeks is shown in Text-fig. 3 D. The main tiller possesses already the primordia of an inflorescence; and

in these the mycelium of the fungus is abundant (Text-fig. 3 D). The mycelium is equally plentiful in the young buds of lateral tillers, and in the nodes and internodes of the main stem. It occurs only sparsely in the leaves.

The fungus produces no visible necrosis in the tissues which it traverses. Whereas the mycelium is almost entirely intracellular during the early stages of invasion, especially in the mesocotyl, during its later development it is largely intercellular with not infrequent cluster of branches, some at least of which are inside the cell (Text-fig. 5 A, B).

B. *The non-virulent strain* (C 3 and C 4). The plants examined correspond in growth with those described in the previous section. The non-virulent strain of smut appears to enter this host with the facility of the virulent strain and after 5 days the distribution of mycelium is similar in the two cases. It is normal in appearance, but perhaps slightly less abundant here than in plants infected with the virulent strain (Text-fig. 4 A).

After 24 days the cases are no longer parallel. Mycelium of the non-virulent strain occurs in the mesocotyl, the stele of which it has penetrated, and in the lower part of the primary node, but it is entirely absent from the tissues above the node and is, therefore, separated from the growing point by a considerable mass of lignified tissue (Text-fig. 4 B). Older plants investigated after 8 and 11 weeks show a more extensive development of mycelium in the stele at the base of the primary node, but as growth proceeds there is an ever widening zone of tissue between the parasite and the apex of the shoot (Text-fig. 4 C, D). The mycelium in the stele tends to break up to form short lengths, but this tendency is seen also in the virulent strain in plants at a similar stage of growth.

IV. DISCUSSION.

The progressive development of mycelium by the virulent strain on the selection of oats used, culminating in the production of spores in the panicle, agrees closely with the descriptions of earlier workers (4, 8). One point of difference is worth noting, namely, the fact that in young seedlings Miss Kolk found mycelium in the space between the first leaf and the coleoptile, and she considers that this is the path of invasion usually followed. I have not found mycelium in this position and conclude that the fungus usually passes from the coleoptile or the mesocotyl to younger organs *via* the primary node.

The behaviour of the non-virulent strain provides for the first time a picture of the successful initial infection of a resistant host followed by a

gradual check to the development of mycelium and, in consequence, the production of normal panicles.

The scope of the investigation did not include a close study of the cytological relationship between host and pathogen, but particular emphasis should be given to the fact that individual strands of mycelium appeared to be equally healthy in the two strains. No special features distinguished the microscopic appearance of either the hyphae of the non-virulent strain or the cells of the host with which they were in contact. No indication was found of encasing sheaths such as Wolff⁽¹⁸⁾ and Lang⁽⁹⁾ have described in other species of *Ustilago*. On this point the results confirm those made on *U. Avenae* in the susceptible oat Victor⁽⁹⁾. The normal appearance of host and pathogen here stands in direct contrast to that which characterises certain forms of rust on resistant varieties of the cereals (1, 2, 3, 10, 13).

The chief interest of the data presented lies in the fact that they refer to the growth of two different strains of smut on the same host. External conditions were maintained as uniform as possible for both classes of plants, and the contrasting results can only be accounted for by the assumption of some difference in the two strains of the parasite. Both succeed in entering the host, but they progress at a different rate, with the result that one passes beyond the stele before hardening sets in, while the other does not. That the so-called non-virulent strain is not lacking in vigour when placed on a congenial host is proved by the results on the variety Grey Winter, which gave 90 to 100 per cent. infection (see (14), Table VI). The histology of invaded plants of Grey Winter was also examined and it agreed with that recorded above for the virulent strain (C 2) on *A. strigosa* (521).

If we attempt to explain the results by assuming say a one-factor difference in the genetic constitution of the two strains of smut which makes C 2 find *A. strigosa* 521 a congenial host, while C 4 finds it uncongenial, we must also suppose that this selection of *A. strigosa* differs from Grey Winter in some factors which enable the former to resist smut C 4 while the latter is successfully invaded by it. Several workers have obtained genetical data which indicate that the inheritance of resistance to smut in oats depends upon the assumption of multiple factors, but the work has seldom been carried out with specified strains of the fungus¹. When these are used it may still be necessary to invoke a number of factors to explain the inheritance of smut resistance in oats, but it is not

¹ For a brief summary of this work and list of references see Cambridge, "Breeding varieties resistant to disease," *Imperial Bureau of Plant Genetics*, p. 18, 1930.

unlikely that some of the seemingly complicated examples may admit of a simpler explanation. The fact remains, however, that we have on the one hand a series of oat varieties differing in factors for resistance to smut, and on the other a series of strains of the smut fungus which consistently differ in their capacity for infection. One expression of resistance seems to be a slowing down of the growth rate of the fungus mycelium.

Recognising the fact that we are concerned with two organisms instead of one, an interesting parallel might be drawn with the phenomenon of incompatibility in higher plants, which appears to be best explained by a theory involving sterility factors which have varied but distinct effects on the growth rate of pollen tubes (5, 6, 15). Since the growth of pollen tubes and the growth of mycelium must have some fundamental features in common it is not inconceivable that similar genes might be involved in both phenomena, namely, the incompatibility between pollen and stigma in flowering plants, and "uncongeniality" in the realm of parasitology.

It seems likely that further microscopic study will reveal different grades of resistance in oats. This result is already foreshadowed in a note on Black Mesdag⁽⁹⁾ and in some preliminary unpublished results which the writer has obtained with the highly resistant variety Markton. If these, and similar observations, can be linked with a genetical analysis of resistance, using as far as possible pure strains of the fungus, they will undoubtedly contribute to a better understanding of resistance phenomena in this group of plant diseases.

V. SUMMARY.

The paper deals with microscopic observations on the invasion of a selection of *A. strigosa orcadensis* by two biological species of *U. Kolleri*, which differ by nearly 100 per cent. in their capacity for producing smutted panicles on this host. Under certain prescribed experimental conditions both strains enter the oat plant with equal facility, but one is retarded in development and fails to reach the growing apex before the tissues of the primary node become lignified, with the result that the panicles produced are not infected.

The results are discussed in relation to the problem of the inheritance of resistance to smut in oats.

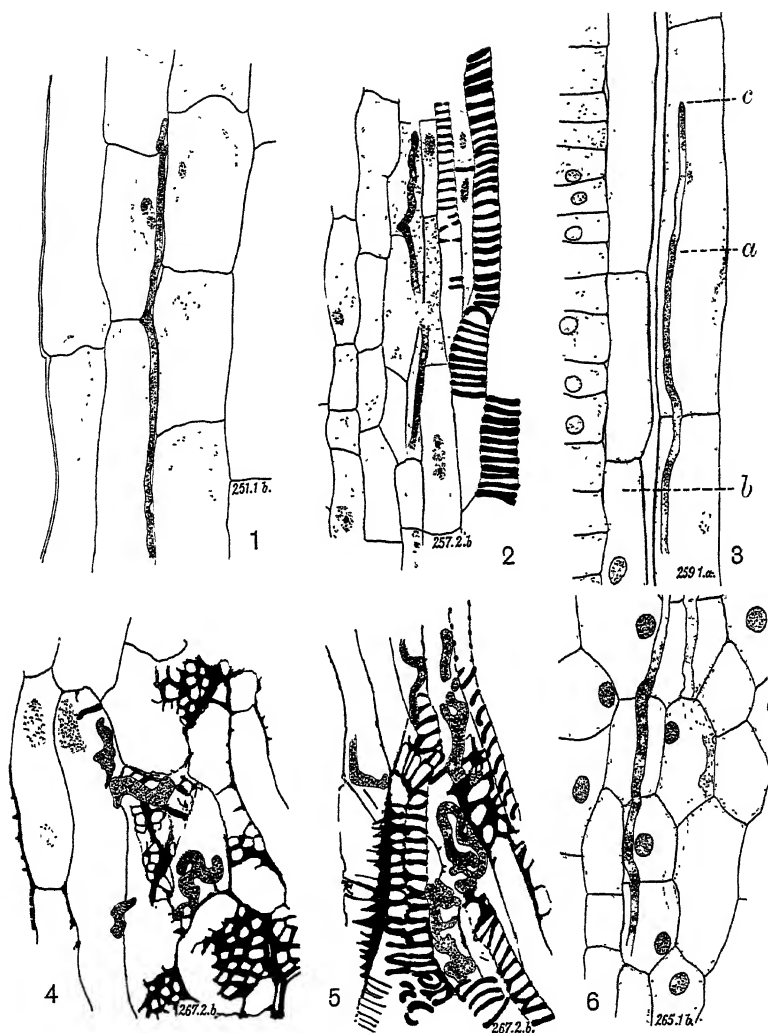
VI. ACKNOWLEDGMENTS.

The writer desires warmly to thank Prof. R. G. Stapledon for granting her the use of greenhouses belonging to the Welsh Plant Breeding Station and for his interest in the work.

Thanks are also due to Mr J. W. Watkins for his careful attention to cultural details.

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EXPLANATION OF PLATE XVI.

All drawings have been made in outline with the aid of a camera lucida. Figs. 1-5 were drawn under a 4 mm. obj. and oc. 15. Magnification $\times 500$. Fig. 6 was drawn under 1/12th obj. and oc. 10. Magnification $\times 760$.

All the figures represent material of *A. strigosa orcadensis* (521) infected with two biological species of *U. Kollerii* which are described as virulent (C 2) or non-virulent (C 3 or C 4) according to their ability or failure to produce chlamydospores in the panicle (see p. 260).

Fig. 1. Coleoptile of seedling 5 days old infected with virulent smut (C 2).

Fig. 2. Vascular strand of the primary node in a seedling 5 days old infected with the non-virulent smut (C 3).

Fig. 3. Same seedling: (a) epidermis of coleoptile; (b) epidermis of first leaf, and (c) tip of a hypha which is intracellular.

Fig. 4. Oat plant, 11 weeks old. Mycelium of non-virulent strain (C 4) among the pitted elements of the stele in the primary node.

Fig. 5. Same plant showing mycelium in the stele at the point of origin of a root.

Fig. 6. Oat plant, 11 weeks old. Mycelium of virulent strain (C 2) in the primordia of a panicle. The mycelium is here intercellular.

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FUSARIUM SPECIES ON BRITISH CEREALS

FUSARIUM NIVALE (FR.) CES. (= ?*CALONECTRIA*
GRAMINICOLA (BERK. & BR.) WR.).

By F. T. BENNETT, B.Sc., PH.D. (LOND.).

(*Advisory Mycologist, Armstrong College, University of
Durham, Newcastle-upon-Tyne.*)

(With Plates XVII-XX and 3 Text-figures.)

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INTRODUCTION.

IN 1929 the writer encountered on a Cumberland farm a complete failure of perennial rye-grass (*Lolium perenne*) in a seeds mixture and, in 1931, when the same field was sown with oats, failure of this crop also, the stand being very thin and most of the plants dying back after reaching a height of about 12 in. The primary cause of both failures proved to be *Fusarium* disease. Four species of *Fusarium* were isolated from the basal parts of the grasses and oats, viz. *F. culmorum*, *F. avenaceum*, *F. graminearum* and *F. nivale*. The relative pathogenic activity of the first three species was known from previous investigations, but that of the last one was not known. On the Continent *F. nivale* has long been recognised as a serious pathogen; Ihssen in 1910 proved that the disease, "Snow Mould," was due to this fungus, and later, Schaffnit(10) described the organism and disease in detail. Atanasoff(1) stated that in Europe it is one of the important cereal pathogens, causing Seedling Blight, Foot Rot, and Head Blight, and that it occurs in the U.S.A. to some extent. Dobrozrakova(4) reported that in parts of Russia it was the sole pathogen attacking

autumn-sown wheat and rye in 1928, from the attacks of which many plants died and infected areas were clearly visible as thin patches. The present writer had found *F. nivale* on barley grain grown in Northumberland and on grasses of golf greens in Northumberland and Durham in 1929 and 1930. It occurred, however, only in conjunction with other pathogenic fungi or under very abnormal conditions, and its identity, as a pathogen, with the Continental strain could not be gauged by observation. The fact is now thoroughly established that in many (or most) species of *Fusarium* there are distinct strains that differ consistently in cultural characters, parasitic capacity, and the pathological symptoms they produce in the host. The writer's isolants of *F. nivale* from barley, oats and grass resembled each other in morphological and physiological features, but differed in these respects from the Continental strain¹, hence there was the possibility that the British and Continental strains might differ in pathogenicity also. In order, therefore, to ascertain what part this organism played in the observed failure of the oats and the rye-grass, and to determine its importance from an economic point of view, it has been studied in some detail by the methods and under the conditions applied by the writer to other species of *Fusarium* (2).

SOME CULTURAL CHARACTERS OF *F. NIVALE*.

When *F. nivale* is the sole or the dominant organism present, as it sometimes is in "Brown Patch" of turf and on roots of rye-grass, aerial mycelium is freely and rapidly developed when the affected material is placed in a moist chamber in a warm room. Under similar conditions infected cereal seedlings very rarely yield obvious mycelium, while grains from infected ears of cereals have never done so under the conditions adopted for germination tests. Such infected material bears, after some weeks, sporodochia or pseudopionnotal patches on the dead tissues, always minute, and recognisable only by the aid of the microscope when similar in colour to the substratum (as on grains), but visible with a hand lens when of normal salmon colour. The method of recognising *F. nivale* depends, therefore, to some extent upon the nature of the infected material. The first growth of aerial mycelium usually bears abundant conidia; from these, and from later formed sporodochia, single-conidium cultures were secured for the following investigation. The later growth of aerial

¹ Prof. Schaffnit's isolation, received from the Centraalbureau voor Schimmelcultures, Baarn. A note stated that the organism did not form spores, nor did it do so when received from Dr Schaffnit two years previously. The writer induced the production of sporodochia by the method mentioned on p. 274.

mycelium is usually sterile, either as a uniform layer or as massive clumps. Wheat-meal agar and oat agar are the artificial media giving the best normal growth; on synthetic media a different type of growth arises, this being particularly useful for differentiation of strains. Single-conidium cultures on wheat-meal agar show the following characters:

Aerial mycelium. Well developed, loose or dense, white but assuming a faint salmon tint in good light; later forming a thin felt on which clumps of sterile mycelium develop, these becoming more frequent the longer the organism is cultivated. Sub-cultures from the mycelial parts eventually yield a non-sporing type.

Sporodochia. These develop slowly on the thinner parts of the aerial mycelium, and on the submerged plectenchymatic layer, whence they push through to the surface of the felted layer; minute when single, or up to 2 mm. when aggregated to composite masses; in colour, at first salmon, then salmon-orange, finally rufous. Partial pseudopionnotes arise in plate and slant cultures, the delicate spore-bearing hyphae disappearing and leaving the spore masses on the surface of the medium.

Conidia. Typically 1- or 3-septate, occasionally 2-, very rarely 4- or 5-septate; broader in the lower half and becoming narrower upwards to a sharp, slightly curved apex; apicellate or with minute attachment process. The conidia show variations in shape under cultural conditions, sometimes being of uniform width except towards the apex, or being elongated and narrower towards the base as in most species of *Fusarium* (Text-fig. 1). In sporodochia 3-septate up to 95 per cent. with measurements as below; 1-septate, 15–20·8 × 2·5–3·5 μ . In pseudopionnotes frequently much modified (Text-fig. 1, VI). On aerial mycelium mostly 1-septate, sometimes 3- and occasionally 4-septate, but of less characteristic form than sporodochial conidia.

Measurements for 3-septate conidia from various sources; average in microns.

Source	British strain	Continental strain
Sporodochia, on naturally infected barley shoot	23·3 × 3·75	—
Sporodochia, on wheat-meal agar	23·6 × 3·5	32·9 × 4·9
Sporodochia, on the plectenchyma on oat agar	23·7 × 3·56	28·9 × 4·7
Sporodochia, on cooked wheat in moist chambers	21·5 × 3·56	28·5 × 4·75
Pseudopionnotes on barley grains from infected ears	13·0 × 2·8	—
Pseudopionnotes on wheat-meal agar in dishes	22·4 × 3·1	—

Chalamydospores. None.

Perithecia. Imperfect; described later.

The difference in size of conidia between the British and Continental strains is clearly marked, but the difference in appearance is even more marked, the conidial walls and septa of the former being exceedingly thin and delicate, whilst those of the latter are clear and firm. Distinctive features in type (and rate, p. 280) of growth between the two strains are well marked on synthetic media¹.

¹ Prior to the comparative tests, physiological differences due to nutrition were reduced or eliminated by growing both strains for several "generations" on wheat-meal agar in Petri dishes, sub-cultures being made by transfer of small marginal pieces of fungus plus medium. Similar inoculum was used for transfer to the synthetic media. For spore measurements the spore-bearing capacity of the Continental strain was revived when cultures on

Dox's agar. British strain: exceedingly delicate, web-like hyphae on or in the surface, extending rapidly and uniformly over the whole; the plectenchyma just below the surface is uniform, diffuse, not coloured; there is no significant difference on media at pH 5.6, 6.8, or 7.4; the habit is similar on slants and in Petri dishes.

Continental strain: a compact plectenchymatic growth into the medium below the inoculum, but no appreciable surface growth; in Petri dishes, where penetration is restricted, there is some expansion of plectenchyma at the surface as lobulated, or frond-like projections, bearing superficially a sparse, woolly mycelium; at pH 7.4 the aerial mycelium is slightly more abundant and the plectenchyma less massive than at pH 5.6.

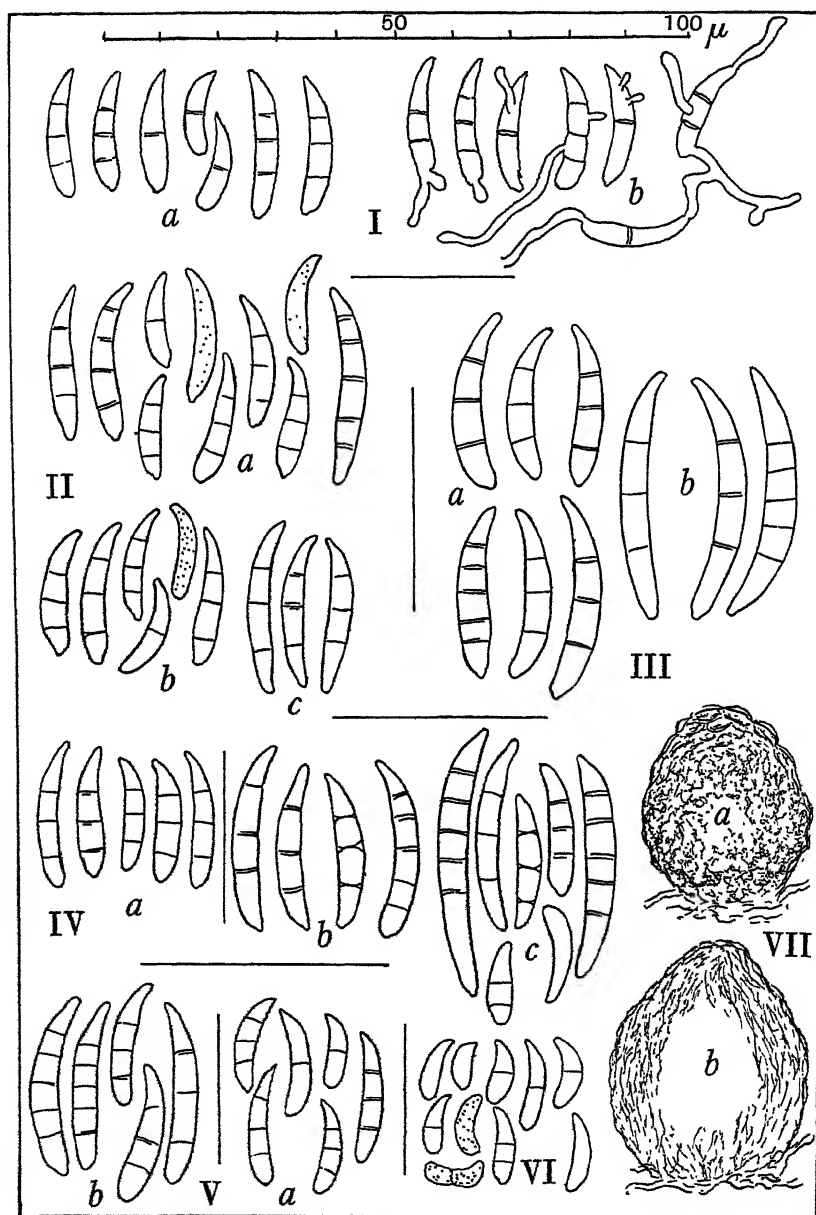
Salts-saccharose agar with citric acid and glyccocoll (p. 279); British strain; growth as on Dox's medium, but showing slight, downy aerial growth on the acid range up to pH 5.5.

Continental strain: on alkaline medium a compact plectenchymatic mass, with frond-like margins and moist or slightly downy surface; on the acid side, the plectenchyma becomes less compact and a short aerial mycelium is obvious.

THE ASCIGEROUS STAGE.

Perithecia have been obtained in immature form only, and in the first growths after isolation from natural sources. They appeared at room temperature, between July and September, on wheat-meal and oat agars, sterilised young stems of lupin, and leaves and grains of oats and rye. Repeated attempts on many kinds of substrata, under varied artificial and natural conditions of temperature and moisture, including low temperatures (p. 278), failed to induce ripening. Wollenweber¹ states that about 10 per cent. only of the isolations of *F. nivale* will produce perithecia, but forms in which this does happen will produce the perfect stage readily on many kinds of substrata. Leonian⁽⁶⁾ has shown for *F. moniliforme* that single-spore selections tend to maintain the characteristics of the form from which the spores are immediately derived. In the present investigation the stock cultures were of single-conidium origin, and from three natural sources. Thus, for two reasons, viz. an insufficient number of isolations, and propagation from single conidia, forms with "ascigerous capacity" may have been missed, and such capacity could not then appear in the cultures unless by purely chance expression of a latent character. This explanation does not, however, completely agree with the actual facts as regards the British strain of *F. nivale*, which fails, not in production, but in maturation of the perfect stage. Cultures from each of the three natural sources have also been grown together on natural and artificial substrata with no better result, thus removing to some extent sterile, cooked wheat grains were transferred to Petri dishes lined with moist filter paper; in from four to six weeks sporodochia were borne on the grains beneath the mycelium, and scattered singly or in small patches on evanescent hyphae on the paper at some distance from the grain masses.

¹ Correspondence (1932) from Dr Wollenweber, with criticisms and suggestions, is gratefully acknowledged.



Text-fig. 1. For explanation see foot of p. 277.

doubt as regards heterothallism. On the Continent *F. nivale* produces perithecia quite commonly, Dobrozrakova⁽⁴⁾ stating that "at the beginning of July pinkish and later light brown perithecia occurred (in fields of wheat and rye), but in pure cultures on *Melilotus* stems the perithecia were always black." What is supposed to have been the perithecial stage of this fungus was found in England in January, 1871, by Berkeley and Broome, on *Aira caespitosa*, and named *Nectria graminicola*; but that this was the present-day *Calonectria graminicola* is very doubtful, and investigation of this point is in progress. Taking all these points into consideration, it is evident that no definite statement as to the presence or absence of perfect perithecia in the British strain of *F. nivale* can be made at present¹.

The immature perithecia in first cultures on wheat-meal agar were ovate, sometimes almost round, the largest ones measuring $170 \times 140 \mu$ to $250 \times 200 \mu$. Corresponding structures, in size from $40 \times 35 \mu$ to $65 \times 50 \mu$, have occurred on sterile lupin stems, and within oat grains that had, after external contamination from pure cultures, been grown on in sterilised soil to seedling stages. The perithecia on wheat-meal agar, though largest in size, were least developed, the peridium consisting of interwoven hyphae, thick-walled but not closely segmented, and of brownish colour by transmitted light. The smaller perithecia, on natural substrata, were blue-black in colour, the peridium consisting of segmented hyphae forming a cellular structure visible towards the apex (Text-fig. 1, VII). The perithecia occurred singly, or in small clusters of three or four on a

¹ Since going to Press positive results have been obtained; these will be dealt with in a subsequent issue.

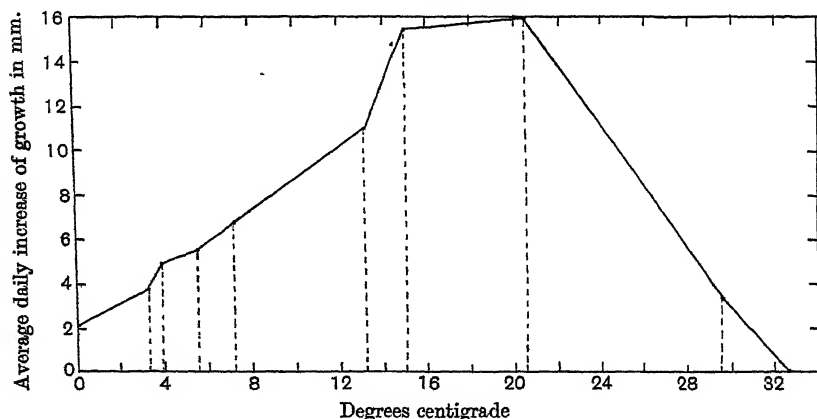
Text-fig. 1. *F. nivale* (Fr.) Ces.; the British strain and some comparisons with the Continental strain.

- I. British strain; conidia (a) from sporodochia on naturally infected barley shoot after being in a moist chamber for 3 months, and (b) the same germinating in water after 24 hours.
- II. British strain; conidia (a) from sporodochia, and (b) from pseudopionnotes in cultures 10 days old on wheat-meal agar; (c) some associated abnormal forms.
- III. Continental strain; grown in parallel with II above; conidia (a) typical forms to compare with II (a), and (b) some associated abnormal forms, from sporodochia in cultures 10 days old on wheat-meal agar.
- IV. British strain (a), and Continental strain (b), (c); conidia from sporodochia in cultures 18 days old on hard oat agar; (c) abnormal forms associated with (b).
- V. British strain (a), and Continental strain (b); conidia from sporodochia on sterile, cooked wheat grains.
- VI. British strain; conidia from infected barley grains after incubation in moist chambers.
- VII. Immature perithecia from pure cultures on (a) lupin stem, and (b) wheat-meal agar (not to scale; see above).

common plectenchymatic base, amongst the hyphae of the felted mycelium on artificial media, or directly on the surface of the natural substrata mentioned.

OPTIMUM TEMPERATURE FOR GROWTH: OVERWINTERING.

In the absence of the perfect stage and of chlamydospores, *F. nivale* persists through winter as dark-brown, plectenchymatic aggregations of mycelium embedded in plant residues, but the aerial mycelium itself is not harmed or modified by low temperatures though it is "fretted" away by exposure to atmospheric conditions. Grains from barley ears inoculated during growth yielded the fungus in the following spring after



Text-fig. 2. Relationship between temperature and rate of growth of *F. nivale*.

Mean temperature	0.0	3.3	3.9	5.5	7.2	13.2	15.0	20.5	29.5	32.5
Average minimum	0.0	1.9	3.1	5.0	6.0	12.4	14.0	20.0	29.0	32.0
Average maximum	0.1	4.7	4.8	6.0	8.4	14.2	16.0	21.0	30.0	33.0

storage at room temperature and also after being outdoors throughout winter. Pure cultures on sterile, cooked wheat grains, after submission to -20°C . on eight occasions at intervals of 5 days and at no part of this period reaching 0°C ., grew vigorously again at room temperature, both on artificial media and on the original substrata transferred to moist chambers. The temperature at which the organism ceases to grow has not been ascertained. At a constant temperature of $0-1^{\circ}\text{C}$. plate cultures showed an average daily increase in diameter of 2.1 mm., with a definite aerial mycelium of white, lanate hyphae, somewhat raised in a peripheral direction. The Continental strain, under parallel conditions, increased by 1.25 mm. daily—little more than half the rate of the other—but with

aerial mycelium more abundant, dense and woolly. The rate of growth increased with increase of temperature up to an optimum of 20–21° C., beyond which it decreased until it stopped at 32·5° C.; after 5 days at this temperature the organism had lost vitality entirely, and had perhaps done so earlier. This temperature is occasionally exceeded in hot seasons; for example, there are records of 33° C. in the shade and 40° C. in the sun in the north of England, and of 54° C. in the sun in the south. At such temperatures all exposed parts of *F. nivale* would speedily be destroyed, and its prevalence in the following season probably reduced in consequence.

The above figure shows clearly that *F. nivale* grows most vigorously at summer temperatures—the average minimum and maximum summer shade temperatures are 12–13° C. and 18–19° C. respectively. Statements that the fungus is a “low temperature organism”, and that it “enjoys conditions of low temperature” are misleading. It certainly makes definite growth at 0–1° C., but so do several other species of *Fusarium* (e.g. *culmorum*, *avenaceum*, *graminearum*, *Scirpi*); they all, however, thrive much better at summer temperatures. The popular name of “Snow Mould” applied abroad to the Continental strain of *F. nivale* has reference to the comparatively abundant mycelium produced on and around diseased plants whilst under snow, without signifying that such environmental conditions are more favourable to the fungus. Similarly, the “Brown Patch” of lawns and golf greens seen between October and February in England is not a winter disease in the sense that *F. nivale* is then more vigorous; the trouble is then more obvious owing to lack of new growth in the grasses. The natural meteorological conditions most favourable for development of all phases of *F. nivale* are those of a moist summer.

GROWTH IN RELATION TO HYDROGEN-ION CONCENTRATION.

The method of investigating growth on artificial media in relation to hydrogen-ion concentration is based upon that of MacInnes (7), the details and methods being modified as follows:

Nutrient solution. MgSO₄, 1 gm.; KCl, 1 gm.; FeSO₄, 0·02 gm.; NaNO₃, 4 gm.; saccharose, 30 gm.; in distilled water to 1500 c.c. To required quantities add granular agar at 3·5 gm. per 100 c.c.

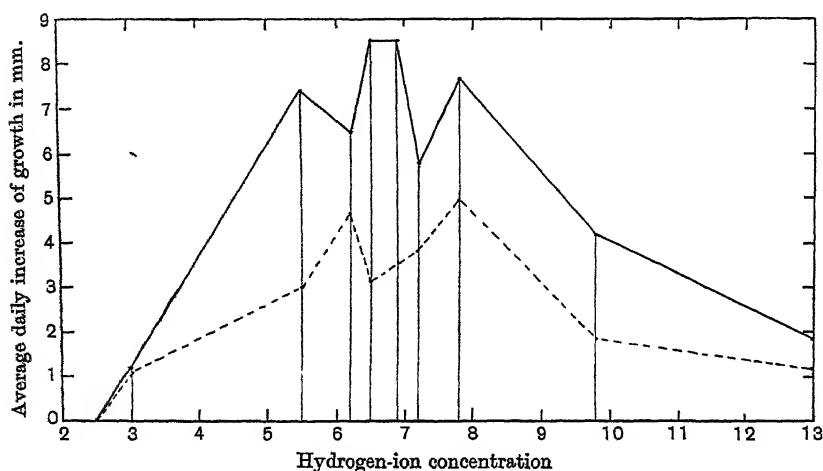
Buffer solution. Citric acid, 4·1 gm.; glycocoll, 1·5 gm.; KH₂PO₄, 1·8 gm.; in distilled water to 200 c.c.

Sodium hydroxide. Seminormal solution.

	Quantities in c.c.											
Nutrient agar	6	6	6	6	6	6	6	6	6	6	6	6
Buffer solution	1·5	1	1	1	1	1	1	1	1	1	1	1
Sodium hydroxide	0	0	1	2	3	3·5	4·5	5	6	7	7	7
Distilled water	6·5	7	6	5	4	3·5	2·5	2	1	0	0	0
pH on mixing above	2·5	3·0	5·5	6·2	6·9	7·2	7·8	9·8	11·0	13·0		

The components, in separate test-tubes, are sterilised at 5 lb. for 30 min., then mixed at about 60° C. to prevent decomposition of sugar at one extreme and to permit setting of agar at the other. Six tubes of each give four sets of the complete medium for Petri dishes and two sets for estimation of pH value. Allowing for occasional contamination, growth tests can be made in triplicate. Each dish (of identical size) will contain 14 c.c. of medium with 1.5 per cent. of agar. The above-mentioned quantities give a suitably wide range but not a constant pH value, owing to such variable factors as acidity of distilled water, composition of tube glass, etc.

When this medium contains less nutrients than mentioned above the growth of *F. nivale* becomes erratic, and variants (or dissociants) occur, whilst with stronger solutions abnormal plectenchymatic forms are developed. The tests were made for a range of hydrogen-ion concentration



Text-fig. 3. Relationship between rate of growth and hydrogen-ion concentration on medium of constant composition. — British strain. - - - Continental strain.

of from 2.5 to 13.0, the other factors being constant—quantity of medium (14 c.c.), size of dishes, temperature (varying between 14° and 16° C.), inoculum used (see p. 274), and absence of light. The average daily increase in diameter, up to a maximum of 18 days, was taken as a measure of the rate of growth, this being shown graphically in the above figure.

For the British strain the rate of growth between pH 5.5 and 7.8, though fluctuating, is of similar order, but beyond these limits it decreases rapidly; the limit for growth on the acid side is about pH 2.5, and on the alkaline side somewhat beyond pH 13.0. The optimum (taking into consideration several trials) is from pH 6.6 to 6.9. The rate of growth does not decrease uniformly from the optimum on either side, since

secondary maxima occur at pH 5.5 to 7.8. Growth over such a wide range of hydrogen-ion concentration, and the occurrence of secondary maxima, are features not peculiar to *F. nivale*. MacInnes(7) found approximate limits of pH 3.0 and 11.7 for an unidentified *Fusarium* from wheat ear; double maxima are recorded by Hopkins(5) for *Gibberella Saubinetii* at 4.0 to 4.5 on the acid side and at a pH not ascertained on the alkaline side. The practical application of these results is referred to later in connection with pathogenicity.

The Continental strain, under identical conditions, differed in type of growth as previously described (p. 275), while correlated with this was difference in rate of surface and submerged growth. Throughout the range of hydrogen-ion concentration the growth of this strain was slower, and showed only two maxima, differing but little, at pH 6.2 and 7.8. When this medium contained salts in the proportion of Czapek's normal solution, the Continental strain made no appreciable growth in three weeks, whilst the British strain, between pH 6.5 and 7.8, showed an average daily increase of 3.5 mm.

The results on Dox's agar substantiated the foregoing ones. On this medium, prepared and standardised by the method commonly practised, the British strain increased at an average daily rate of about 11 mm. at both pH 5.6 and 7.4—equal rates at these pH values as on the former medium. Under parallel conditions the Continental strain increased at the rates of 1.6 and 3.5 mm. respectively—again in accord, but with different ratio, with the former results.

It must be mentioned that the foregoing results follow the "planting" of a small piece (2-3 mm. in diameter) of inoculum of the type specified. Conidia, on the contrary, do not germinate in strongly acid media; for example, these spores germinate and grow well in malt gelatine of pH 5.5, but at pH 4.8 germination is completely inhibited. The limit for germination of conidia, approximately pH 5.2, is markedly different from that for growth of established mycelium, and this difference holds good for both British and Continental strains.

The general inference is that whilst the British and Continental strains agree in some respects, *e.g.* limiting factor for germination of conidia, and range of hydrogen-ion concentration throughout which mycelial growth continues, there are clearly marked differences in the rate and habit of growth at the different pH values on the various synthetic media used. These physiological differences, together with the morphological differences specified (p. 274), suffice for the definite distinction of the strains by cultural methods.

PATHOGENICITY IN RELATION TO SOIL REACTION.

The investigation of growth of *F. nivale* on artificial media in relation to hydrogen-ion concentration was intended to furnish some guidance in the preparation of soils of different hydrogen-ion concentrations, so linking up laboratory and field work. Most cultivated soils in this country have pH values between 5.5 and 7.8. As previously shown, *F. nivale* thrives almost equally well anywhere between these limits, hence it might be inferred that it will flourish in practically all our cultivated soils so far as acid or alkaline conditions are concerned. This assumption, however, would not be justified, since recent research shows that other factors may have an influence under natural soil conditions. Schaffnit and Meyer-Hermann⁽¹¹⁾ state that the Continental strain grows best at 7.9, and is unable to grow in acid soils, and this may have some connection with the facts demonstrated by Sanford and Broadfoot⁽⁹⁾, viz. that the pathogenicity of *Ophiobolus graminis* is more or less suppressed in the presence of certain soil organisms; it is possible, therefore, that the existence and pathogenicity of other fungi (e.g. *F. nivale*) might be modified in the soil by the antagonistic action of the natural micro-flora. Hence conclusions of practical value can be drawn from actual pot and plot experiments only. A further factor that might influence the results of pathogenicity experiments has been demonstrated by Leonian⁽⁶⁾, who has shown that for a single species of *Fusarium* (*F. moniliforme*), different isolations gave forms differing in pathogenicity; further, dissociants from a single-spore culture showed differences in pathogenicity from severe to nil, but, upon repetition of the experiment, the order of pathogenicity might be entirely reversed. In other words, for any one isolant a number of tests must be made, or a number of isolants must be used together, in order to avoid errors due to variability of pathogenicity that may accompany variability of morphological and physiological characters. In the present investigation precautions were taken to avoid errors from the causes mentioned. For soil and seed inoculations the growths from isolants from different sources were mixed, whilst the soils used were sterilised and unsterilised lots in parallel from both field and garden.

The soils used were loams from garden and field, both containing considerable vegetable residues, that in the field soil being from grass roots only. Soil was sterilised by autoclaving, then exposed outdoors, miles away from any cereal crop, for one month or more in order to recover. The hydrogen-ion value was adjusted to the required degree of acidity by sprinkling a heap with dilute sulphuric acid, and of alkalinity by adding dry, air-slaked lime. Treated and untreated (control) lots were left outdoors, the heaps twice turned, for three or four weeks (sheltered from heavy rains only) in order to come to stable condition before estima-

tions of hydrogen-ion concentrations were made (quinhydrone electrode method). Leaching by overwatering was avoided.

Cultures from single conidia from naturally infected hosts of three kinds (p. 273) were maintained separately. For soil inoculation such separate cultures on sterile, cooked wheat were mixed, and some of the mixture added to each pot; for controls an equal amount of sterile, cooked wheat was used.

All grain for seed was soaked overnight in water at about 16° C., disinfected externally with formalin, kept in sterile, moist dishes, and planted when good germination was assured; "misses" could not then be attributed to non-viable seeds.

It is of fundamental importance from a practical point of view to ascertain whether or not the British strain of *F. nivale* agrees with the Continental strain in being restricted to soils with alkaline reaction. In the experiment made for this purpose the soils were adjusted to pH values of 4.5, 7.1 and 8.9. The soils in the pots were inoculated by mixing inoculum in the upper layer of each, watered, and kept covered with glass. A vigorous growth of mycelium developed on the exposed inoculum, and on three occasions at intervals of 6 days this surface layer was turned under to a depth of 2 or 3 in. Thus for nearly three weeks the fungus was subjected to the influence of acid, neutral and alkaline soil conditions respectively. Wheat, barley, oats and rye were then planted, at the end of February, and grown on in an unheated, but warm, greenhouse for eleven weeks. In all the inoculated soils all four cereals were of rather poorer growth than in the corresponding control soils, indicating a general check to growth in the former. The differences were not, however, sufficiently marked to be worthy of illustration. Wheat showed failure of a number of seedlings, and rye a distinct check to growth in the alkaline, contaminated soils; oats, on the other hand, showed markedly poorer growth in the acid, contaminated soil, whilst barley did not differ from the controls under acid or alkaline conditions. The fungus was found to be established on the underground parts of plants in all three soil groups. It existed in moribund and dead parts only, such as seminal roots and coleoptiles, but was never found in vigorous roots or in shoots. The effect on the growth of the plants showed that the fungus was not significantly more pathogenic in soil of one reaction than another. The evidence was conclusive that the British strain of *F. nivale* persists in soils within, and even beyond, normal limits of acidity and alkalinity, and within this range it is not seriously pathogenic to the early stages of any of the four cereals, providing conditions favour reasonably rapid germination of seed and continued growth of seedlings.

The results were distinctly different when cereals were grown under unfavourable conditions, secured by keeping the pots after planting in a

partially shaded place exposed to cold winds and low temperatures (including night frosts) during April and May, and keeping the soil moist continuously. The effect on oats in soils at pH 5.5, 6.8, 7.8 and 8.5, and on barley at pH 6.0 and 7.7, was observed under these adverse conditions, and the results are illustrated in Plate XVII, figs. 1-4. The seedlings in all inoculated soils were distinctly poorer than in the controls, indicating a general check on the early growth by the fungus; in addition, there was failure of oats to the extent of 20 per cent. in soil at pH 5.5, and greater loss of barley (60 per cent.) in soil at pH 6.0. In order to ascertain the subsequent effect upon the plants, and whether there was recovery from initial attacks, one of the duplicates was left under the said adverse conditions whilst another was placed in a sunny position and the soil kept drier; the plants were reduced to six in number in all pots so as to permit full growth. Under the adverse conditions the plants in inoculated soils developed more slowly than the controls, and were poorer plants at maturity, but no Foot Rot or Deaf Ear symptoms occurred. Plate XVIII, fig. 5, illustrates the mature stage of the same oats as shown in Plate XVII, fig. 1, and typifies the general results, viz. that the check upon seedlings in contaminated soil persists throughout subsequent growth but does not cause failure of plants. The plants grown on under better conditions, apart from being slightly later, could not be distinguished from the corresponding controls.

The conclusions drawn from these results were substantiated by wheat experiments in good soil at pH 7.4. The soil was inoculated two weeks prior to planting. When braided, one set was placed outdoors (November) and another set in a warm greenhouse. The latter in the six-leaf stage (end of March) showed frequent discoloration of coleoptiles, but no attack on living stems or tillers, and only slight check in growth as compared with the controls. The outdoor plants were badly discoloured at the bases, but had not died off. A duplicate set then brought into the greenhouse (end of March) continued growth, but plants in contaminated soil remained poorer in stem, height and vigour than corresponding controls. The duplicates left outdoors to grow to maturity are illustrated in Plate XVIII, fig. 6; they show that in contaminated soil the plants remain poor in all respects, but without Foot Rot or Deaf Ear phases. It may be assumed that if winter conditions had been more severe than those of 1931-2 the fungus would have caused more serious damage.

Conclusions. All the results justify the conclusion that *F. nivale* in soils may, under conditions unfavourable for plant growth, attack the shoots of germinating seed, cause Seedling Blight at braiding stage, or

cause a permanent check on the growth of plants affected basally. These effects are, for oats and barley, most marked in acid soils, but are not restricted to them. The British strain can exist, and attack any of the cereals, in all normal field soils whether of acid or alkaline reaction. Under good growing conditions, since the fungus is unable to attack vigorously growing tissues, no cereals suffer serious damage from it, and plants affected in their earlier stages (if not too severely) recover more or less completely under favourable weather and soil conditions.

PATHOGENICITY IN RELATION TO INFECTED SEED CORN.

Artificial infection of ears: Ear Blight. The method of infection was as follows:

The inoculum consisted of an aqueous suspension from pure cultures on wheat-meal agar of such conidia and mycelial fragments as passed through a single layer of cheese-cloth; conidia were abundant, and in water drops germinated freely within 12 hours. Such material taken from a culture derived from a single conidium is called "monospore inoculum," and that prepared by mixing from several single-conidium cultures "multispore inoculum"; the inocula were tested in parallel. For controls the inoculum was replaced by tap-water. The inoculum was applied as drops between spikelets and rachis and to the exterior of all spikelets. The treated ears were enclosed in glass tubes with a plug of wet cotton-wool in the opening, and kept so covered in a shady place for 6 days; in field plots the ears were covered for 5 days and were exposed to sunlight. A second method of inoculation was the spraying with inoculum of ears (usually about 18) of all plants in one pot and leaving them uncovered, misty nights and damp days (Sept.) providing the requisite moist conditions.

The ears of the three varieties of wheat, three of barley, and two of oats, that were tested, showed susceptibility to attack by *F. nivale* under conditions of artificial infection; the fungus was isolated from naturally infected barley also. Younger ears are less liable to infection than older ones, especially in barley, not because they are less susceptible but because of failure of inoculum to adhere to the more waxy surface. The lesions resemble those caused by other common species of *Fusarium*, and are exhibited as reddish-brown dots marking the points of infection, extending later to bleached areas with pale-brown margins or with scattered brownish markings when adjacent patches coalesce. The discoloration is well marked on green barley ears, but much less so on wheat ears; in fact, infected wheat ears would escape even careful observation in a field crop. Ripe ears, when dry, simply show a dingy colour due to the brownish marks as compared with healthy ears. The fungus establishes itself most frequently at the point of attachment of grain to rachis, where moisture is best retained, and such infection about flowering time prevents subsequent development of grains. When infection occurs on the glumes or pales these are discoloured as mentioned, but grain develop-

ment is neither inhibited nor retarded. These effects are illustrated for barley in Plate XIX, fig. 7; the Ear Blight there shown is typical for both monospore and multispore inocula, which showed no significant difference in virulence. Such ears, after storage under cool room conditions from October to April, showed the following percentages of obviously infected grains from the different inoculation groups: controls, 0; exposed to casual infection, 15; sprayed with inoculum under outdoor conditions, 35; multispore inoculum and kept moist for 6 days, 89; monospore inoculum and kept moist for 6 days, 100. Thus, by simple application of the fungus to ears, or merely exposing them to infection, during damp weather, Ear Blight was pronounced, whilst under artificial conditions of continually damp atmosphere it was disastrous. The attack on the grains was, however, confined to the non-living parts, the embryo remaining unaffected, as revealed by the following tests. First, grains from the above groups, after external disinfection, tested in the usual way showed germination percentages varying between 75 and 84, *i.e.* there was no significant difference between apparently sound grains and obviously infected ones. That the fungus was present and in viable condition in the discoloured, though germinating, grains was proved by the consistent occurrence of the conidial stage (Text-fig. 1, VI) on their external surfaces in the germination dishes. Secondly, fifty grains from each of the groups were planted from the germination dishes into good soil, and all yielded seedlings that remained vigorous and to all appearance healthy up to the four-leaf stage. At this stage ten seedlings from each group were transplanted to good soil and grown on under good conditions, and to poor soil and grown on under poor conditions. All continued growth, so the plants were reduced to seven and six per pot in the good and poor soils respectively, for growth to maturity. The result is illustrated in Plate XIX, fig. 8; infected seed gave plants of slower growth, less vigour, and fewer ears than did healthy seed, with the differences more marked in the plants grown under poor conditions.

Ear Blight in wheat, apart from the difficulty of discerning affected ears, is similar to that in barley. Inoculation by standard methods, and in parallel, showed Iron, Little Joss and Red Marvel to be equally susceptible. Examination of grains and subsequent growth has not been done in detail as for barley, but other experiments indicated that the results would be similar.

Artificially contaminated seed. Seed corn was inoculated as follows:

After external disinfection the seed was dipped into a very heavy suspension of multi-spore inoculum, then transferred to sterile Petri dishes lined with moist filter paper, and kept until germination was evident; viable seeds were planted therefrom.

Inoculated grains, with parallel controls, were planted in natural, untreated soils, the duplicate controls sufficing to indicate the absence of pathogenic organisms, and grown on under poor conditions as previously mentioned. The plants from contaminated seed were, in every instance, poorer than those from healthy seed (Plate XX, figs. 10-13). In wheat and rye the stems were thinner, the leaves narrower and paler in colour, and the general vigour lower; oats and barley showed similar effects but to less extent. Under conditions of moist soil and partial shade during subsequent growth, the initial check resulted in poorer plants at maturity, as shown in Plate XVIII, fig. 6, but less marked than when plants are in contaminated soil. The results of planting externally contaminated seed corresponded exactly to those following planting of infected seed from blighted ears.

Under parallel conditions the Continental strain of *F. nivale* proved more strongly pathogenic than the British strain. Of the inoculated seed in the Petri dishes many failed to germinate at all, and germination of the others was seriously retarded. When germinating seeds were planted they gave, for all four cereals, plants inferior to those contaminated with the British strain, especially for rye, oats and barley (Plate XX, figs. 11-13). The differences in the plants—withered leaf-tips, paler coloured and flecked leaves, early withering of lower leaves, and generally lesser vigour—gave every indication that these plants, if grown more slowly to maturity, would have suffered more seriously than those tested with the British strain.

Conclusions. *F. nivale* attacks the ears of cereals under moist conditions, preventing grain formation or development when the rachilla is invaded, and causing discoloration of glumes, pales and pericarps when the grains themselves are attacked; but it does not damage the embryo, and thus does not reduce the germination capacity of the grain harvested. It reduces the value of the crop partly by reduction in yield of corn, but more so by discoloration of the sample. Seed infected whilst in the ear, or contaminated externally subsequently, sown in clean soil yields poor crops but not failures; the inferiority of the crops is more obvious when they are compared with healthy crops, i.e. those differing only in absence of infection. Good growing conditions very largely counteract the disadvantages of contaminated seed.

PATHOGENICITY IN RELATION TO TWO SPECIES OF GRASSES.

The question of the pathogenicity of *F. nivale* towards certain grasses is of interest. Sampson (8) has reported the occurrence of this fungus in the "Brown Patch" of golf greens in Britain, and Dahl (3) states that cereals and certain grasses have been infected in greenhouse experiments by *Fusarium* spp. occurring on lawns and golf courses in the U.S.A. and Canada. The former investigator did not examine *F. nivale* for pathogenicity, and the latter did not name his fungi specifically. The question, therefore, remains undecided. The writer can, as yet, report preliminary results only in this respect, with reference to perennial rye-grass (*Lolium perenne*) and Italian rye-grass (*L. Italicum*), the failure of which was one reason for the present investigation. On both garden and poor field soils in their natural condition, heavily contaminated on and in the surface layer with multispore inoculum (p. 285) in the form of pure cultures on cooked wheat, these grasses showed no reduction in "take." The subsequent growth, however, was retarded, and the "stand" distinctly poorer than in uncontaminated soil (Plate XIX, fig. 9), and more so on poor than on good soil. The grasses were also narrow in leaf and pale in colour, with withered leaf-tips and leaves that gave the impression of a "wilt." After 2½ months' growth the grasses were cut back; the second growth showed that the fungus was seriously affecting the plants by girdling the stems at soil level and killing them. Perennial rye-grass suffers more than the more vigorously growing Italian variety, and the damage is actually more severe to both grasses in the good garden soil, contrary to first impressions; this is doubtless due to the fungus thriving better in the more open soil containing considerable decaying vegetable matter. It is anticipated that, in consequence of the continued activity of the fungus on the plants whilst growth is at a standstill during winter, the rye-grasses will die out completely next season.

SUMMARY.

1. *F. nivale* (? *Calonectria graminicola*) was commonly present along with one or more other species of *Fusarium* on the basal parts of oats and perennial rye-grass that had failed in a field. The pathogenicity of the other species of *Fusarium* being known from previous investigations, that of *F. nivale* remained to be ascertained.

2. In wheat, barley, oats and rye, this fungus causes some loss of plants before or after brairding, and it acts as a continuous check on the growth of established plants sown and grown under adverse conditions,

whether infection arises from contaminated soil or seed; good growing conditions largely counteract the effects of contamination.

3. *F. nivale* also attacks ears and grain, preventing grain formation or causing discoloration of formed grains; whilst possible thus to reduce the yield and market value, such attacks are rare in the field.

4. The chief result arising from contaminated soil or seed in practice is a general reduction in vigour, a condition more likely to be attributed to causes other than the true one.

5. Perennial and Italian rye-grasses on contaminated soils are reduced first in growth; subsequently, the former is largely killed off by the end of the season, but in the field this stage would be reached in the second season.

6. *F. nivale* grows vigorously within the range of normal summer shade temperature, its optimum being 20–21° C.; it grows slowly at 0–1° C., and in its vegetative state withstands winter conditions, as well as exposure to temperatures as low as – 20° C.; it ceases growth, and loses vitality within a few days, at 32–33° C.

7. In culture the fungus grows on media of hydrogen-ion concentration from 2.5 to 13, the optimum being 6.5 to 6.9; its conidia do not germinate in media more acid than pH 5.0 to 5.2. Neither the existence nor the pathogenicity of the fungus is appreciably affected by the acid or alkaline condition of soils within ordinary field ranges.

8. Comparative cultural studies of the British and Continental strains of *F. nivale* show that they differ consistently and significantly in size of conidia, and in form and rate of growth on certain selective media. These morphological and physiological differences are accompanied by difference in pathogenicity, the Continental strain, so far as has been tested, being more virulent toward cereals and less virulent toward grasses than the British strain.

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EXPLANATION OF PLATES XVII—XX.

PLATE XVII.

- Figs. 1–3. The effect on oats of *F. nivale* when present in soils of pH values 5.5, 6.8 and 8.5. The fungus exists and is pathogenic to oats in acid soil.
- Fig. 4. Illustrating the pathogenicity towards barley in soil of acidity such as commonly occurs in fields.

PLATE XVIII.

- Mature plants when grown in soil contaminated with *F. nivale*; in general, less vigour, and indirectly poorer crop and yield.
- Fig. 5. Oats; the final stage of the plants illustrated in Plate XVII, fig. 1, six plants left.
- Fig. 6. Wheat, as grown outdoors from November to August; contaminated soil (left) has greater adverse influence than contaminated seed (right).

PLATE XIX.

- Fig. 7. Ear Blight of barley inoculated artificially soon after emergence of ears from the sheaths; showing sterile spikelets and discoloured grains.
- Fig. 8. Discoloured (infected) seed from inoculated ears, and seed from uninoculated ears of the same plants, grown on under good and poor conditions respectively; infected seed gives poorer plants and fewer ears, especially when grown under poor conditions.
- Fig. 9. Perennial and Italian rye-grasses; showing the poorer first growth in soil contaminated with *F. nivale*; subsequent deterioration is progressive.

PLATE XX.

- A comparison of the pathological effects of the British and Continental strains of *F. nivale*.
- Fig. 10. Wheat; the British strain inducing poor thin stems and leaves, but not crippling the plants; the Continental strain causing definite check and dying back.
- Fig. 11. Rye; the relative effects are similar to those on wheat.
- Fig. 12. Oats; the British strain causing poorer growth in stem and leaf; the Continental strain rendering the plants worthless.
- Fig. 13. Barley; on this host the British strain is much less pathogenic than the Continental strain.

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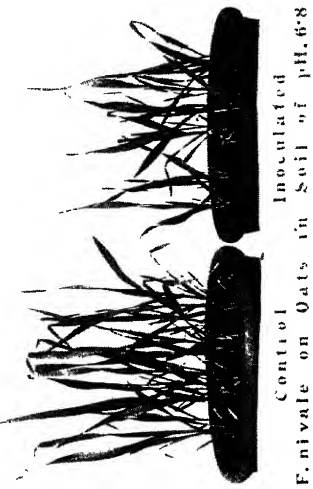


Fig. 1.



Fig. 2.



Fig. 3.

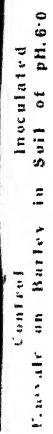


Fig. 4.

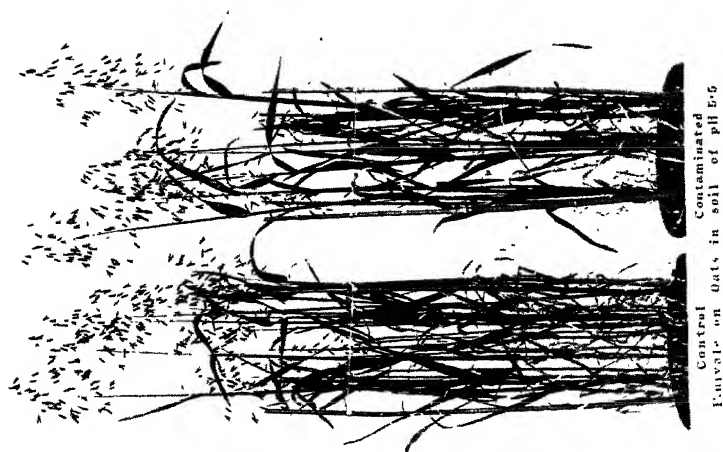


Fig. 5.

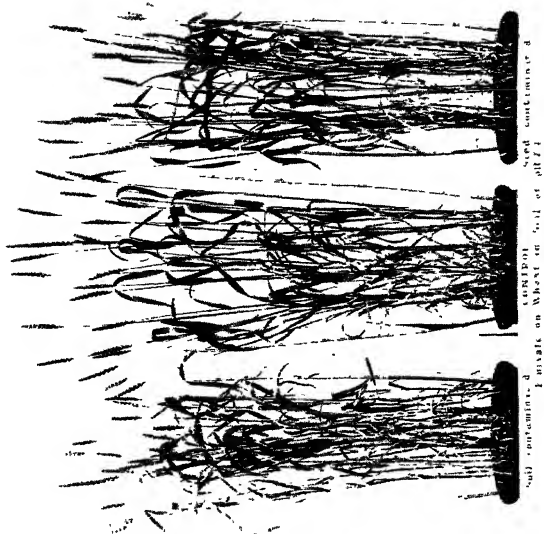


Fig. 6.

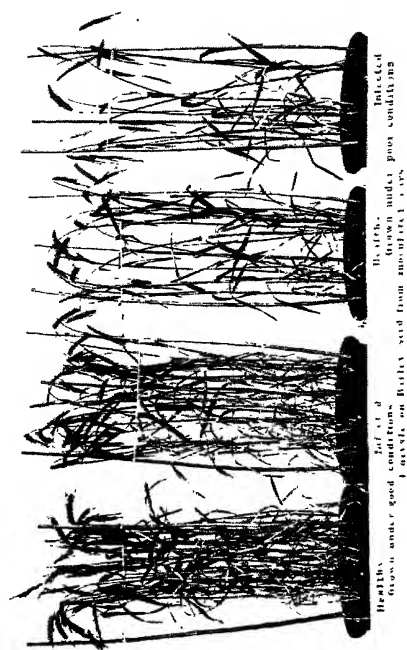


Fig. 8.



F. nivale

Fig. 7.



Fig. 9.

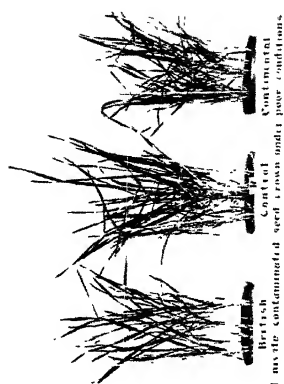


Fig. 10.

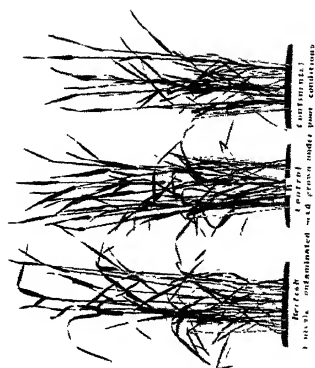


Fig. 11.

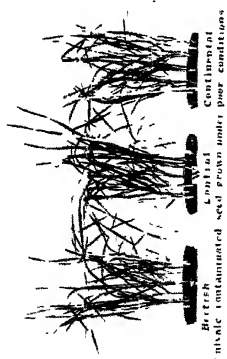


Fig. 12.

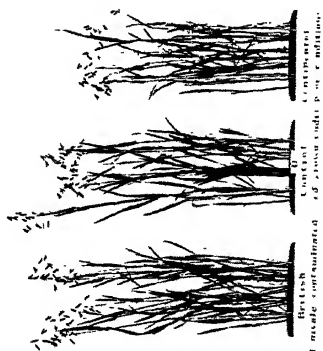


Fig. 13.

SOME ASPECTS OF THE PHYSIOLOGY OF CERTAIN NITRITE-FORMING BACTERIA

BY D. WARD CUTLER AND L. M. CRUMP.

*(From the General Microbiology Department,
Rothamsted Experimental Station.)*

DURING the course of work carried out for the Department of Scientific and Industrial Research, as part of the programme of the Water Pollution Research Board, on the purification of waste waters from a beet sugar factory by biological filtration it was found that comparatively large quantities of nitrite were produced in the filters. A large number of bacterial strains, which had been isolated from such filters, were therefore tested upon media containing various ammonium salts to see whether among them there were any which could convert ammonia into nitrite. It was found that out of 229 species of bacteria which were tested 104 produced nitrite from ammonium salts to some extent, and in behaviour were very similar to those already isolated from soil by Cutler and Mukerji(1).

The results given in this paper were obtained from a survey of the whole bacterial population which was carried on over two seasons, and detailed results regarding individual species and their morphological and physiological characters are not in a sufficiently advanced stage in the majority of cases to be given here.

The filters at the beet sugar factory were in general principle similar to those used in sewage work and the medium of which they were constructed was either gravel or clinker. Infection from the air could easily occur, and the waste water from the factory also contained large numbers of micro-organisms from the soil which was introduced with the roots of the sugar beets. The usual practice in such factories is to concentrate the work into a short period of 90 to 100 days, and there is therefore a long period from the end of one season to the beginning of the next when the filters stand dry and idle. Further descriptions of the filters and of the biochemical changes which the solution undergoes in its passage through them are given by Jenkins(2) and Barritt(3). The nitrifying bacteria in question appear to be very catholic as regards their reactions; the same species may be capable not only of forming nitrite but also of

causing it to disappear, nitrite may also be formed from urea, and by the reduction of nitrate, nor have the conditions under which these changes take place yet been definitely determined. It is obvious therefore that much work remains to be done, but at the same time a preliminary communication outlining the types of behaviour exhibited may be of interest to those working on similar problems.

METHODS.

In the course of routine examination of the bacterial strains one of the tests employed was to inoculate the cultures into ammonium sulphate in mineral salt solution¹ to which 0.1 per cent. of different carbohydrates had been added. The carbohydrates were sucrose, dextrose, laevulose, lactose, maltose, galactose and mannitol. The formation of acid and gas was recorded, and the nitrite present was estimated by the Griess-Hosva method (4).

Those cultures giving a nitrite reaction were then further tested on the following additional ammonium salts: phosphate, carbonate, acetate and lactate.

RESULTS.

The different strains isolated from the filters fall into three groups, the first comprising those which produced acid and gas on one or more of the carbohydrates, the second those producing acid but no gas, and the third, those giving no reaction of this type in spite of their growth. Table I gives the numbers of nitrite and non-nitrite forming bacterial species which produced acid on one or more of the carbohydrates. All species are omitted which only occurred sporadically on the filters.

Table I.
*Numbers of carbohydrates from which acid was formed by
nitrifying and non-nitrifying bacteria.*

Type of bacteria	Number of carbohydrates							
	0	1	2	3	4	5	6	7
Nitrite forming 1st season	15	10	9	5	1	0	1	0
Non-nitrite forming 1st season	19	25	13	17	7	1	2	2
Nitrite forming 2nd season	3	6	3	9	12	16	9	5
Non-nitrite forming 2nd season	3	4	4	5	1	7	9	6

¹ The composition of the medium was as follows: 1.0 gm. $(\text{NH}_4)_2\text{SO}_4$, 0.6 gm. NaCl, 0.02 gm. CaCl_2 , 0.005 gm. MgSO_4 , 0.3 gm. K_2HPO_4 , 1000 c.c. H_2O .

The results of the first season suggest that on the whole the bacteria in the filters were species which were comparatively inert on the carbohydrates, only a small percentage being able to utilise more than four; the second season's work, however, contradicted this idea, although the conditions of infection and of growth were the same in both years. It is interesting that there was no difference to be observed in the behaviour of nitrifying and non-nitrifying bacteria upon carbohydrates, although the nitrifying forms are not usually regarded as organisms which obtain energy from carbohydrate decomposition.

The comparative popularity of the carbohydrates employed is shown in Table II where the numbers include both nitrifiers and non-nitrifiers.

Table II.

Percentage numbers of bacteria forming acid on different carbohydrates.

No acid	Sucrose	Lactose	Dextrose	Galactose	Laevulose	Maltose	Mannitol
17.4	37.1	28.8	44.9	46.7	49.7	34.5	34.8

As would be expected from their place of origin it is a small percentage which are wholly without action on the carbohydrates.

Nitrite formation on different ammonium salts.

The strains were then tested as to their nitrifying power on the following ammonium salts: carbonate, phosphate, sulphate, lactate and acetate, and the results are shown in Table III.

Table III.

Percentage of strains producing 0.4 or more milligrammes of nitrite nitrogen per litre on different ammonium salts.

Carbonate	Phosphate	Sulphate	Lactate	Acetate
0	35.5	16.1	45.1	3.2

From this it is seen that of the salts tested the lactate was the most suitable though phosphate also gave good results. Among the species that have been worked upon in greater detail the following four, P 15, P 30, L 24 and Z 20, have been found readily to form nitrite when urea is the sole source of nitrogen in the culture medium. A similar result in the case of asparagin was obtained by Cutler and Mukerji⁽¹⁾.

The actual amounts of nitrite formed on phosphate, sulphate and lactate are given for certain representative strains in Table IV.

Table IV.

Nitrite nitrogen in milligrammes per litre produced by various strains on ammonium salts.

Strain	Phosphate (days)			Sulphate (days)			Lactate (days)	
	3	5	7	3	5	7	5	7
D 28	0	0	0	0	0	0	0.1	0.8
J 10	0.4	0.8	0.4	0.8	0.4	0.2	0	0
N 18	0.25	0.4	0.8	0.1	0	0	0	0
P 15	0.05	0.4	0.4	0	0	0	0	0
P 30	0.4	0.8	0.8	0.8	0.2	0.1	0.1	0.4
S 37	0	0	0	0	0	0	0	0.8
S 40	0	0	0	0	0	0	0.05	0.8
X 44	0	0	0.1	0.05	0.8	0.1	0	0
Z 20	0.2	0.4	0.4	0.05	0.8	0.1	0.05	0.8

Disappearance of nitrite.

It will be seen from Table IV that the amount of nitrite in the cultures fluctuated from time to time; thus, while it sometimes increased steadily, as in the case of N 18 on phosphate, in other cases there was a sudden falling off in amount, as is seen with P 30 on sulphate. It was decided therefore to test whether the disappearance was due to the absorption of nitrite by the bacteria, or to its reduction to ammonia. Fourteen strains were inoculated into a mineral salt solution containing 1.6 mgm. per litre of nitrogen as sodium nitrite. The results are given in Table V.

Table V.

Disappearance of nitrite in the presence of various bacterial species.

Mgm. of nitrite nitrogen per litre
(days)

Strain				
	0	3	5	7
J 7	1.6	1.6	0.1	0
J 9	1.6	0	0	0
J 10	1.6	0.3	0.2	0
K 16	1.6	0.4	0.4	0.2
L 24	1.6	1.6	1.6	0
N 16	1.6	0.3	0	0
N 18	1.6	1.5	0.6	0
P 30	1.6	0.8	0.8	0.05
S 31	1.6	0.4	0.4	0
X 14	1.6	0	0	0
X 44	1.6	1.6	1.2	0
Y 12	1.6	0	0	0
Z 13	1.6	0	0	0
Z 20	1.6	0	0	0

At the end of the experiment the solutions were tested for ammonia by the Nessler reaction, and only one strain, L 24, had produced ammonia. These results are similar to those already recorded for soil bacteria by

Cutler and Mukerji(1). Although the possibility that nitrate may be formed cannot be disregarded, there is evidence to show that this did not occur in the experiments in question. It will also be noticed from Table IV that in no case did the nitrite disappear when derived from ammonium lactate. As already mentioned there is reason to believe that this salt is the one from which nitrite is most readily produced, and it is possible that in the case of the other salts the nitrogen present as nitrite is more readily available to the bacteria than when present as ammonia. In the case of the strains from soil investigated by Cutler and Mukerji(1) the same thing was found during the first 9 days on ammonium lactate though afterwards the nitrite disappeared in some cases.

In order to discover if there was any relationship between the numbers of bacteria and the amount of nitrite found, a series of experiments were set up and the bacterial numbers were counted and the nitrite estimated daily. For this purpose four species of bacteria were selected. The results of these experiments are given as a contingency table (Table VI), where the initial growth period, that is the time from the inoculation to the first maximum, is contrasted with the later period where the bacterial numbers are fluctuating.

Table VI.

Correlation of bacterial numbers with nitrite production for species L24, P15, P30, Z20; bacterial numbers given first (a + sign signifies an increase, a - sign a decrease).

	++	+-	-+	--
Growth period	68	15	—	—
Later period	38	57	60	75

For all species during the initial period nitrite formation and increase in numbers are closely correlated; but in the later period this does not occur. It would appear that when the cultures have reached maturity the numbers of bacteria and the amounts of nitrite bear little relationship one to another. A certain amount of experimental evidence has been obtained tending to show that the C/N ratio has a decided effect in conditioning the disappearance of nitrite. This fact will undoubtedly have a bearing on the contingency table figures for the later period of growth. In a further paper it is proposed to give the experimental evidence and consider this problem in detail.

A further possibility with regard to the disappearance of nitrite arises in some cases. The strains already referred to in Tables IV, V and VI do not produce acid in the course of growth, but where species are used which give rise to acid conditions in the presence of sucrose, this may

account for the disappearance of nitrite during the later stages of growth. To test this point an experiment was carried out in which a strongly nitrifying species was inoculated into a mineral salt solution containing 0.1 per cent. sucrose at a *pH* of 7.2. After 5 days' growth, when the *pH* value was reduced to 4.8, the nitrite which had previously been formed had disappeared. The culture was then sterilised to kill the bacteria and 0.7 mgm. per litre of sodium nitrite was added. The nitrite was not immediately diminished by the acid conditions, but after 48 hours' standing it had completely disappeared. Therefore in work of this kind it must be borne in mind that conditions may arise in the cultures leading to the disappearance of nitrite without the agency of bacteria; at the same time the disappearance of nitrite when acid is formed in the culture is by no means consistent since during this work many cases have been recorded when the *pH* value was below 5.0 but the nitrite remained.

SUMMARY.

1. One hundred and four species of bacteria which produce small quantities of nitrite from ammonium sulphate have been isolated from filters receiving waste water from a beet sugar factory, and these bacteria do not differ in their behaviour on carbohydrates from non-nitrifying bacteria from the same source.
2. Ammonium lactate is the salt of ammonia which is most readily oxidised.
3. In the majority of cases nitrite can also be utilised by these bacteria in the course of growth.
4. There is a positive correlation between increase in bacterial numbers and the percentage nitrite in a culture during the initial growth period.
5. Nitrite may disappear slowly from solutions at a *pH* of 4.8 when the bacteria have been killed by autoclaving; but this is not invariably the case.

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OBSERVATIONS ON GROWTH IN LARVAE OF *PLODIA INTERPUNCTELLA* HUBN.

BY MARY MILES, M.Sc. (MANCHESTER).

(From the Department of Zoology (Agricultural Entomology),
the Victoria University of Manchester.)

(With 2 Text-figures.)

It has been observed (1, 6, 7) that there is considerable variation in the time required for the completion of the life cycle in *Plodia interpunctella* Hubn. In the author's preliminary investigations this variation in the length of the life cycle was directly associated with differences in the number of larval ecdyses, the rate of larval growth and the duration of larval stadia, and appeared independent of environment. Variation in the number of larval stadia within the limits of a single species, *Arctia caia* L., has been observed by Chapman (3, 4), and Imms (8), discussing it, writes, "The factors determining the number of ecdyses need investigation and it is at present impossible to judge how far they are phylogenetic in significance, and how far they are an expression of internal physiological processes in the species concerned." It was decided, therefore, to investigate more fully larval development in *P. interpunctella*.

The writer wishes to express thanks to Dr H. W. Miles for his continued interest in the work and for his helpful suggestions in matters of technique; to Dr G. H. Carpenter for reading the manuscript, and to the late Prof. J. S. Dunkerly for his interest in the earlier part of the work.

METHODS.

In an initial study of larval growth it was essential that larvae under observation should be permitted to mature under similar environmental conditions. This consideration limited observation to those external features which could be used as indications of development: the number and duration of larval stadia and the rate of growth.

The choice of a suitable means of expressing the amount of growth at ecdysis was a matter of difficulty since weighing and measuring single active larvae was impracticable. In the following observations the width of the head capsule in successive instars has been used to indicate the amount of growth at ecdysis. The ratio of the width of the head in successive instars does not express absolutely the ratio of increase in size at ecdysis; but, since growth in one part of the body is directly associated

with growth in other parts, measurements of the head capsule in successive instars may be used for purposes of comparison. This character of corresponding growth was recognised by Dyar⁽⁵⁾ who found that there was a direct association between the width of the head capsule and the instar in larvae of Lepidoptera.

At ecdysis the head capsules of larvae of *P. interpunctella* were usually shed entire. The head is not subject to growth during the stadium so that the width of the cast head capsules of successive instars represents the amount of growth at ecdysis. At the final larval ecdysis the head capsule was split along the epicranial and frontal sutures and so distorted as to render accurate measurement impossible. The width of the head capsule in the final larval instar is, therefore, not available.

The larvae were separated into series for observation, each series having the same provenience. A comparison of growth in different series would indicate the influence of the origin of the larvae on the rate of growth and number of ecdyses.

To obtain larvae for observation, four pairs of newly emerged adults were isolated. No details of the biology of the adults were known. They were selected at random from a stock which had bred in captivity for over a year. Eggs were obtained from each pair and on hatching the larvae were placed separately in small phials containing thin slices of walnut. Twenty-four larvae of the same provenience were used to constitute a series for observation.

The larvae were maintained at a constant temperature of 21° C. Approximately the same amount of food was available for each larva and a sufficient quantity was provided so that the larvae were never prevented from feeding by a lack of food. No observations were made on the humidity conditions, but, since the phials were not air-tight and were all placed in the same compartment of the incubator and contained practically the same amounts of food material, it was considered that the humidity was a factor constant for all the larvae. As evidence that slight differences in humidity which may have arisen during the investigation had no significant effect on the data collected, it may be said that variation in the duration of larval life was quite as apparent in mass cultures of larvae of the same provenience as in larvae reared in isolation.

The phials containing the larvae were examined daily and records kept of the duration of the larval stadia and the number of ecdyses. The food was renewed at frequent intervals and the insects kept isolated until the emergence of the adults. In this way accurate data were obtained on the number and duration of the larval stadia and the sex of the imagines.

NUMBER AND DURATION OF LARVAL STADIA.

Fig. 1 indicates the number and duration of larval stadia in four series of larvae.

Data concerning number of stadia are as follows: of 74 larvae, 10 had five stadia, 52 had six stadia and 12 had seven stadia. This suggests that

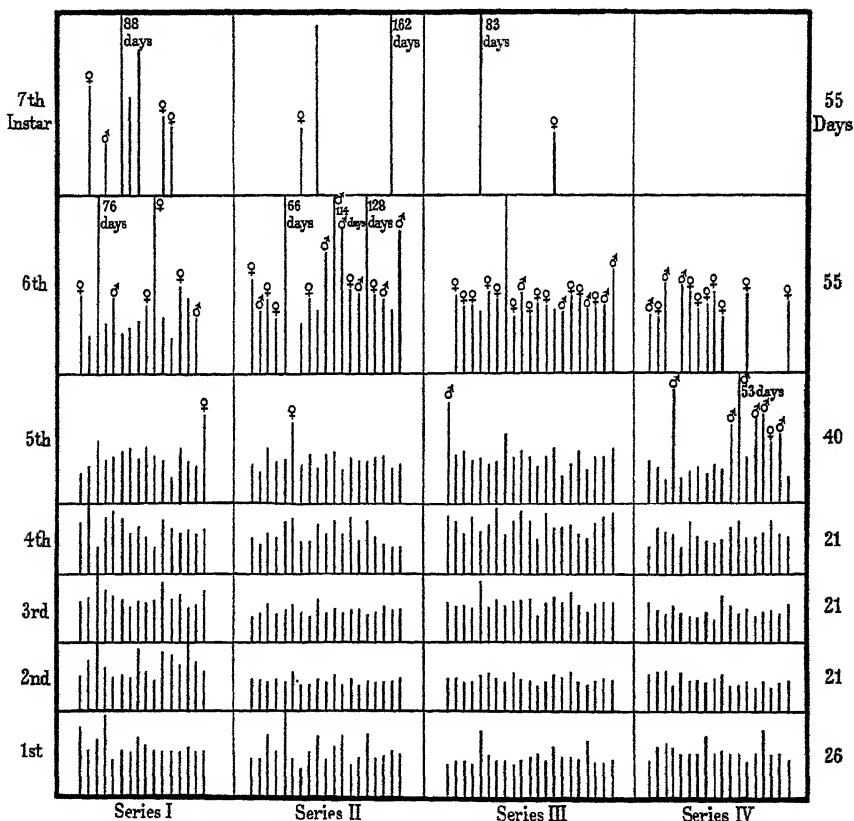


Fig. 1. To show variation in number and duration of larval instars in *P. interpunctella*.

six larval stadia is usual for *P. interpunctella* when fed on walnut kernels and maintained at a temperature of 21° C. Of the 12 larvae having seven stadia only six attained imaginal development; the rest died after a more or less prolonged seventh stadium. No larva having five larval stadia failed to reach maturity, while only five larvae out of 52 having six larval stadia failed to attain imaginal development.

300 *Observations on Growth in Larvae of P. interpunctella*

There is some evidence that length of larval life is associated with number of larval stadia. Larvae having five stadia pupated after 64 to 99 days, with an average of 76.1 days of larval life. Larvae having six stadia pupated after 69 to 182 days, with an average larval life of 85.7 days. Out of 47 six-stadia larvae attaining imaginal development only three spent more than 99 days as larvae, and the omission of these three would reduce the average length of larval life in six-stadia larvae to 82.5 days. The occurrence of seven larval stadia seemed to be associated with some retardation of growth for the length of larval life in larvae having seven stadia varied from 84 to 120 days, with an average of 107 days. The total duration of larval life for insects reaching maturity varied from 64 to 182 days.

Differences in the time required for larvae to attain the physiological condition associated with ecdysis were marked. The duration of the first larval stadium varied from 9 to 25 days, the second from 7 to 21 days, the third from 7 to 21 days, the fourth from 8 to 21 days, the fifth from 7 to 53 days, the sixth from 11 to 114 days and the seventh from 16 to 34 days in larvae attaining imaginal development. The greatest variation occurred in the ultimate larval stage where the minimum duration was 17 days and the maximum was 114 days.

There are considerable differences in development in the series of larvae. Series I, II and III have each only one larva with five instars, while in Series IV there are five. Larvae with seven larval instars occur in Series I, II and III, while in Series IV there are no larvae having seven instars. Larval development was most rapid and regular in Series IV.

Dyar⁽⁵⁾ and Chapman⁽³⁾ concluded that the sex of the imagines had an influence on larval development, larvae becoming females usually having an extra ecdysis. The data collected during this investigation showed no clear relationship between the sex of the imagines and the number of larval stadia. In Series IV, however, most of the larvae having five instars were males while the majority having six instars were females, and most of the seven instar larvae in the remaining series were females. This suggests that the sex factor cannot be entirely ignored as a cause of variation in the number of larval stadia in *P. interpunctella*.

Observations on the final larval stadium seemed to indicate that its prolongation was associated with some retardation of vital physiological processes. In the following table some details are given concerning larvae with a prolonged final stadium.

Table I.

Larva	No. of larval stadia	Duration of final larval stadium (days)	Attained imaginal development
5	7	34	Yes
7	6	76	No
11	7	88	"
13	7	45	"
15	6	57	"
6a	6	66	"
11a	7	52	"
13a	6	37	"
15a	6	114	Yes
17a	6	44	"
20a	7	128	No
23a	7	162	"
24a	6	44	Yes
5b	7	83	No
8b	6	58	"
24b	6	32	Yes
4c	5	35	"
12c	5	53	"

For the purposes of the above table the final larval stadium was considered prolonged after 30 days. Of 18 larvae having a prolonged final stadium 11 failed to attain imaginal development, while of 55 larvae with a normal final stadium only one died in the larval stage. This suggests that the prolongation of the final larval stadium is an indication of some retardation in the physiological processes. From a study of larval development it appears that the occurrence of a prolonged final stadium had some association with the rate of development. Thus, of ten larvae having five instars, two had a prolonged final stadium; of 52 larvae having six instars, nine had the final stadium prolonged, and of 12 larvae with seven instars, seven had the final stadium prolonged. This suggests that the factors causing the final stadium to be prolonged are similar to those which tend to increase the number of larval stadia. The likelihood of surviving the prolongation of the final stadium seems also associated with the rate of development. Both five-instar larvae having a prolonged final stadium attained imaginal development; of nine six-instar larvae having a prolonged final stadium, four attained imaginal development, while of seven seven-instar larvae with a prolonged final stadium only one attained imaginal development. These observations seem to indicate that the number of larval stadia and the duration of the final larval stadium are directly associated with physiological processes.

RATE OF GROWTH IN LARVAE OF *P. INTERPUNCTELLA*.

The rate of larval growth has been indicated by measurements of the width of the head capsule in successive larval instars. The head capsules,

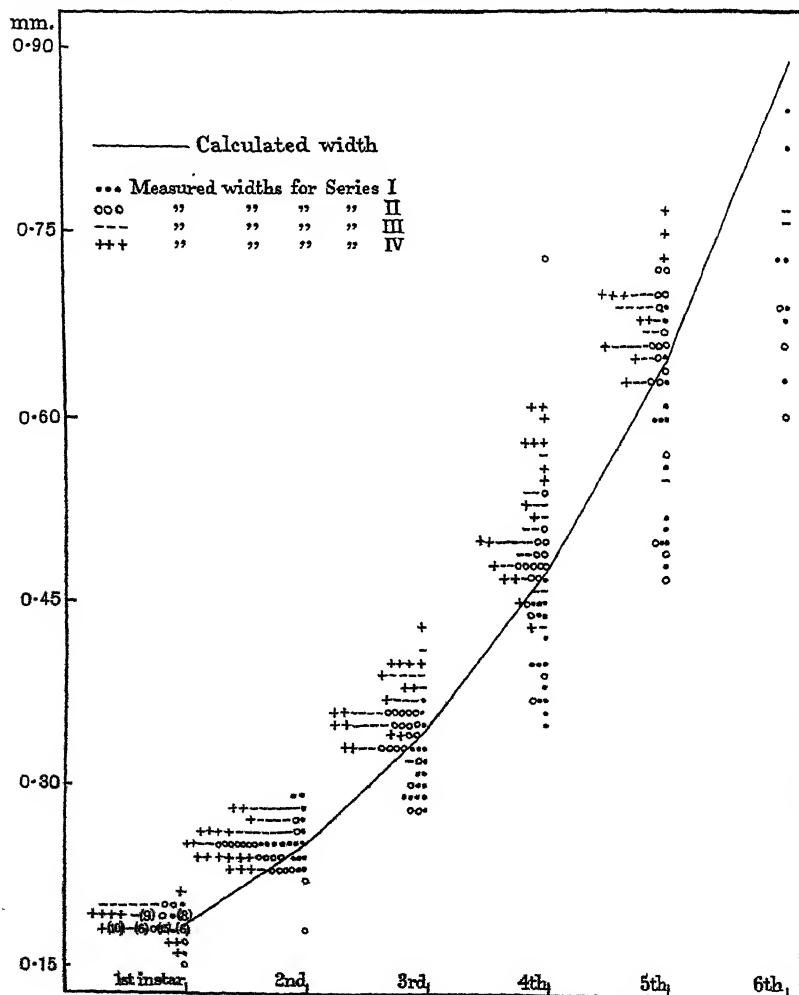


Fig. 2. Width of head capsule in *P. interpunctella*.

which were rigid, were measured across their broadest part with a camera lucida and micrometer scale. The same microscope, eye-pieces and objective were used for the entire series and the scale tested from time to time. Fig. 2 indicates the measurements.

The width of the head capsule of the first instar larvae was fairly uniform, 58 of the 75 head capsules measuring 0.18–0.19 mm. In the second instar variation was greater, only 30 head capsules having the average width, 0.25–0.26 mm. No. 9*a* moulted 9 days after eclosion from the egg but remained the same size after ecdysis. In the third instar variation in the width of the head capsule further increased and only 15 head capsules were of the average width, that is 0.34–0.35 mm. No. 9*a* which made no growth at the first ecdysis increased in size considerably at the second, while No. 7 showed no increase in size at the second ecdysis though the second stadium lasted 21 days.

Variation in the width of the head capsule was greatest in the fourth instar, the measured widths being 0.35–0.75 mm., and only 14 larvae had the average head width of 0.47–0.48 mm. The smallest head width was that of No. 24 whose record is incomplete owing to the head capsule being broken at the third ecdysis. This larva may have had two instars in which the head was practically the same width or it may have increased in size only slightly at the third and fourth ecdyses. No. 7*a* increased in size at the third ecdysis to more than twice that of the third instar; this suggests that an ecdysis was missed, but reference to data concerning the life history showed that the third stadium lasted 11 days, a period less than the maximum, and that there was no instance where the total number of days for the third and fourth stadia was less than 16 days.

The width of the head capsule in the fifth instar was 0.40–0.77 mm., the average width being 0.64 mm. Where the head capsule of the fifth instar was 0.50 mm. or less in width data showed that growth at previous ecdyses had been below the average or that there had occurred ecdyses at which growth was almost inhibited. Where the width of the head capsule was about 0.70 mm. data showed that growth at previous ecdyses had been steady and generally fairly considerable.

Only those larvae having seven larval instars completed the sixth ecdysis, leaving the head capsule entire. Nos. 5 and 23*a* had in the sixth instar head capsules measuring less than the average for the preceding instar, and most of the other measurements were much below the calculated width for the instar.

A study of the head widths in successive instars indicated that there was some association between the number of larval stadia and the amount of growth at ecdysis. A comparison of successive head widths of Nos. 5, 8, 16, 18, 9*a*, 11*a* and 23*a* with those of Nos. 1*b*, 4*c*, 11*c*, 12*c*, 17*c*, 19*c*, 22*c* and 23*c* showed that larvae having five instars were larger at each instar after the first than those larvae having seven stadia. This is

corroborated by a consideration of the head capsules of the fifth and sixth instar throughout the series; in general, larvae which became pupae at the sixth ecdysis were, in the fifth instar, larger than larvae which became pupae at the seventh ecdysis. Nos. 11, 12 and 5*b* were exceptions, but 5*b* increased in size only slightly at the sixth ecdysis and Nos. 11 and 12 failed to pupate.

The calculated growth ratios showed that the rate of growth varied considerably during larval life. In larvae under observation the growth ratio varied from 1.00 where the width of the head capsule showed no increase at ecdysis, to 2.28 where the width of the head capsule after ecdysis was more than twice that of the preceding instar. The greatest fluctuation in the rate of growth occurred in No. 7*a* where the growth ratio of the width of the head at the first ecdysis was 1.15 and at the third ecdysis 2.28. In other larvae, Nos. 12, 9*a*, 20*a*, 24*a*, 3*b*, 8*b*, 9*b*, 12*b*, 13*b*, 1*c*, 12*c*, 24*c*, fluctuation in the growth ratio at successive ecdyses was not more than 0.1.

Dyar(5) found that the width of the head capsule followed a regular geometrical progression in successive instars of larvae of Lepidoptera. The width of the head capsule in successive instars of larvae of *P. interpunctella* was calculated in accordance with Dyar's law, and it was found that the calculated widths were similar to the average of measured head widths for each instar except the sixth. This curve of growth is shown in Fig. 2. The measurements for the sixth instar are those of larvae whose development appeared to have been retarded and which were not, therefore, in the prepupal instar, while the calculated measurement for this instar probably represents approximately the average width of the head in the prepupal instar. It is evident that in *P. interpunctella* where there is considerable variation in the size and number of the instars Dyar's law of growth cannot be insisted upon. A head width of 0.40-0.43 mm. might occur on larvae of the third, fourth or fifth instar or of 0.60-0.73 in larvae of the fourth, fifth or sixth instar, and none of these widths is sufficiently near any calculated width to afford a reliable clue to the instar of the larva.

Data revealed that there was no direct association between the duration of a larval stadium and the amount of growth at the subsequent ecdysis. Where the duration of a stadium was constant for a number of larvae the growth ratio showed considerable variation as, for example, in the case of 10 larvae of the first series which had a first stadium lasting 14 days and a growth ratio at the first ecdysis varying from 1.32 to 1.61. On the other hand, the growth ratio of 5*b*, 7*b*, 8*b*, 13*b* and 21*b* was 1.39 at the third ecdysis though the time passed in the third stadium varied

from 8 to 19 days. In a number of instances the prolongation of a stadium beyond the usual period was followed by sub-normal growth at ecdysis, though in a few cases a fairly prolonged first stadium was followed at the first ecdysis by an increase in size above normal. A stadium of short or normal duration was occasionally followed by an extraordinary increase in the width of the head; thus the fourth stadium of No. 7 lasted 8 days and the growth ratio at the fourth ecdysis was 1.86, and No. 7a had a third stadium of 11 days and a growth ratio at the third ecdysis of 2.28.

A comparison of the rates of growth in the different series indicates that besides the occurrence of individual variation some variation may be associated with the series. Growth in Series III and IV is generally more regular than in Series I and II.

DISCUSSION AND CONCLUSION.

In his study of the development of *A. caia* L. Chapman (4) found that the larvae could be separated into three types: Forwards, Normals and Laggards, according to the rate of development, number of ecdyses, succession of plumage and habits of hibernation. The great mass of larvae were Normals, but Forwards and Laggards were present in every brood. He concluded that variation in the number of ecdyses was of phylogenetic significance; that the Forwards were a bivoltine race closely intermixed with the univoltine Normals and Laggards, and that the races could be separated by breeding and selection.

Chapman's efforts to establish a race of Forwards by breeding were not successful. He found by experiment that the proportion of Forwards could be greatly increased by raising the temperature at which the larvae were reared. It is now recognised (2) that the effect of raising the temperature at which larvae develop is to induce a general acceleration in the rates of their metabolism and consequently in their rates of growth. Differences between Forwards and Normals in succession of plumage and habits of hibernation also seem to be the reaction of larvae to differences in temperature conditions. Forwards maturing before the onset of low temperatures were not exposed to the conditions which induce hibernation and the colour and plumage associated with hibernating forms as were the more slowly developing Normals and Laggards. From this consideration of Chapman's data it seems that variation in larval development is not necessarily the result of racial differences within the species.

Tutt (10), considering Chapman's data and conclusions, regards the phenomenon of variation in the number of larval ecdyses as a provision

of nature for guaranteeing the continuance of the species under extraordinary weather conditions. The variation of 6-14 stadia in larvae of a single provenience is, he says, "a simple matter of variation and not of disease." Larval health and vigour seem best indicated by the subsequent attainment of imaginal development. Chapman does not appear to have made observations on the number of larval ecdyses in relation to imaginal development, therefore there is no evidence that the slowly developing Laggards were not affected by disease.

Hamblin, Reed and Phillips(6), working on the biology of *P. interpunctella* on raisins, prunes and figs, suggest that the variation in the duration of larval life may be correlated with such differences in the fruits as sugar and moisture content. In this investigation differences in the rate of larval development seemed independent of food. Each larva received only a small portion of food at a time, in the early stages less than a quarter-inch square cut thinly with a razor blade. Half a walnut kernel was sufficient to feed a considerable number of young larvae for some days, and at all times during the investigation numbers of larvae were feeding on portions of the same walnut.

From this study of the development of the larvae of *P. interpunctella* it is concluded that the rate of larval growth, the number of larval stadia and the duration of larval life are interrelated. Collected data show that marked increase in size at ecdysis, reduction in the number of larval stadia and in the duration of larval life are associated with the vigour necessary to pass successfully the several phases of metamorphosis. On the other hand, a large proportion of larvae making little increase in size at ecdysis and having an increase in the number and duration of the larval stadia die without attaining imaginal form. This seems to indicate that variation in the number of larval stadia, rate of larval growth and duration of larval life are the direct results of acceleration or retardation of physiological processes.

SUMMARY.

Studies on larval growth in four series of *P. interpunctella* of different provenience, maintained at 21° C. and fed on walnuts, have shown that:

(a) Six larval stadia was usual but development might be accelerated so that larval life was completed in five stadia or retarded so that seven stadia were required.

(b) Number of larval stadia was associated with rate of growth at ecdysis, larvae growing rapidly at each ecdysis having fewer stadia than those growing more slowly.

(c) Duration of larval life was associated with number of stadia and rate of growth at ecdysis, five-stadia larvae tending to complete larval development in less time than six-stadia and seven-stadia larvae.

(d) Duration of the final larval stadium has considerable influence on duration of larval life, and the tendency for the final larval stadium to be prolonged was most pronounced in larvae having seven stadia.

(e) Prolongation of the final larval stadium was frequently followed by failure to attain imaginal development.

(f) Rapid growth and reduction in number and duration of larval stadia are associated with the vigour necessary to pass successfully the several phases of metamorphosis.

(g) Variation in number of larval stadia, rate of larval growth and duration of larval life are the result of acceleration or retardation of physiological processes.

Full details of number and duration of larval stadia, measurements of the width of the head capsule in successive instars and the growth ratios for 74 larvae have been filed at the Department of Economic Entomology, British Museum.

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BIOLOGY AND LIFE HISTORY OF *LIMOTHRIPS CEREALII* HALIDAY AND *APTINOTHRIPS RUFUS* GMELIN FEEDING ON GRAMINEAE

BY U. S. SHARGA, M.Sc., Ph.D., F.E.S.

(From the Department of Agricultural Zoology,
University of Edinburgh.)

(With Plate XXI and 1 Text-figure.)

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I. INTRODUCTION.

LIMOTHRIPS CEREALII has been known in Great Britain as a pest of cereals, especially wheat, for many years. The communications by Marsham (1797, 1798) to the Rev. Samuel Goodenough show that this insect was doing appreciable damage to the wheat crop in the middle of the eighteenth century.

Kirby was the first to detect the insect and he called it *Thrips physapus*. In a letter dated August 27th, 1795, he mentioned his findings on the nature and extent of damage to the wheat crop. According to him this insect was responsible for considerable damage to wheat that year.

Haliday (1836) described it as *L. cerealium*. According to him the wheat crop in England in 1805 suffered immensely, and at Piedmont in the same year about one-third of the wheat crop was destroyed. The contemporary journals of that year give some idea of the outbreak (Haliday, 1836). Since then *L. cerealium* has been recorded at various localities in Britain by Williams (1913), Bagnall (1910), Morison (1928) and others.

Miles (1921) found that the pest damaging cereals in 1918 in England was probably *L. cerealium* or *L. denticornis* which caused sterile spikelets in oats. Theobald (1922) found that *L. cerealium* and *L. denticornis* seriously damaged wheat in Kent and occasionally oats and other cereals also. Since then there has been no observation published on this pest in this country.

Abroad it has a very wide field of distribution and is found as a pest of cereals in France, Italy, Germany, Austria-Hungary, Norway, Sweden, Rumania, Russia, Holland, Denmark, Finland, Spain, Australia, United States of America, Hawaii, Egypt, India and North Africa. Recently Körting (1930), working on the biology and relationship of thrips to plant diseases, considers that *L. cerealium* and *Haplothrips aculeatus* are not responsible for the "white-ear" disease in rye, oats or barley, and probably not in wheat, though further tests with this crop are needed.

Aptinothrips rufus is a common grass-inhabiting species and is found throughout the year. In the absence of any previous work on this species, a study of the habits and life history was made in the summer of 1930, and the details of seasonal distribution of larval and adult forms and variations in the fecundity of the two forms were studied by collections made from grass fields near Edinburgh throughout the year.

The work was done in the Department of Agricultural Zoology, University of Edinburgh, under the supervision of Dr C. B. Williams. I have great pleasure in thanking him for suggesting this piece of work and for valuable advice received during its progress; and also for placing at my disposal his collections and literature on thrips. I also wish to thank Dr G. D. Morison for copies of his papers on thrips and for the loan of the very rare male of *A. rufus* var. *stylifera*.

II. BREEDING TECHNIQUE.

Breeding of *L. cerealium* was done in the departmental greenhouse. Wheat seedlings were brought from fields when 4-5 in. high and planted in flower pots with one seedling in each pot. Females were liberated at the bases of leaves and a study of feeding, oviposition, and other habits of the adult was made in this way.

When the larvae hatched from the eggs, they generally moved inwards into the leaf-sheaths. The larvae after hatching were therefore removed and placed on leaves having leaf-sheaths attached to them, and these were placed in Petri dishes lined with wet blotting paper. The blotting paper was kept moist and the covers of Petri dishes were kept slightly open so that excess of moisture should not prevail. This method caused great mortality and was abandoned later in the year when ears of wheat were available.

At this time medium-soft calyces were removed from the ears and each newly hatched larva was placed into one of them, which were placed on a wet blotting paper forming the lining of petri dishes. When the calyx is removed from its position the outer margin curls down a little and gives a little shade to the feeding larvae inside, and also leaves a little space open above for examination purposes. The outer surface of the calyx becomes wet in 2-3 days and remains succulent and juicy for a longer period. The calyces were changed every 4 or 5 days until the larvae were in the resting pupal stages. In cases where a female oviposited in a fresh calyx, the egg and larval periods were observed in the same material.

The breeding of *A. rufus* was tried in summer on grass seedlings sown in small specimen tubes. Larval stages were observed on grass blades or sometimes were placed in wheat calyces where moulting and other habits were observed as in *L. cerealium*.

III. *LIMOTHIPS CEREALIMUM* HALIDAY.(i) *Emergence from hibernation.*

Only adult females of this species survive the winter. The factors that seem to determine the emergence from hibernation are increased sunlight and warmth. The meadow grass near the wheat fields at Liberton and Boghall Experimental Farm was examined by sweeping from April onwards. No thrips were found in April. The examination of wheat seedlings also showed that they were free. Thrips were first found in small numbers on May 21st, 1930, at Liberton, Edinburgh.

The dissection of these thrips showed that they had immature ovaries. From this time onwards increasing numbers of thrips were captured in every sweep from meadow grass till, by the middle of June, they were present in enormous numbers.

The emergence of thrips at Boghall Experimental Farm was later and specimens were first taken from meadow grass on June 3rd. Evidently meadow grass is the first plant on which the emerged thrips are found.

(ii) *Oviposition.*

Thrips first captured had no mature eggs. The dissection of eighteen females on May 26th revealed that in two the eggs had begun to develop. By June 4th all the wheat plants were heavily infested with adults. In some cases eggs were also found embedded in the leaf-tissue near the leaf-bases.

After the infestation of wheat plants, thrips were invariably found at the bases of leaves, and consequently in the beginning they oviposited chiefly at the leaf-bases. Later in the season, oviposition spread all through the plant. The females continued egg-laying for a considerable time and the eggs were laid from the apex to the base of leaf, inside the leaf-sheath or in the calyx. On oats the oviposition was greater inside the leaf-sheath and calyx, and much less in leaves.

In the laboratory no definite time for oviposition was noticed, but generally more females were seen ovipositing in the morning than in the afternoon. The process of oviposition is as follows: the female keeps on alternately feeding and moving till a suitable spot seems to have been selected. It then arches the abdomen slightly and brings the ovipositor to an angle and forces it up to the base into the leaf-tissue. The ovipositor seems to penetrate the leaf-tissue obliquely. After moving the ovipositor forwards and backwards into the slit, the female becomes motionless and some movement at the tip of the abdomen shows that the egg has passed into the slit. The time required is 2 min. and only one egg is laid in each pocket.

If the leaf be examined against light, the eggs appear as small white spots. Some eggs are laid inside, others more superficially, occasionally even with the end projecting.

(iii) *Hatching.*

The eggs when laid are whitish and later change to brownish with two visible red spots (the eyes of the larva).

When the larva is formed, it breaks through the chorion, the head with two bright red eyes being the first part to emerge. The larva then

moves backwards and forwards and occasionally sideways with slight jerks. It emerges little by little with each movement, until only the tip of the abdomen remains in the egg.

When the greater part of the body has come out, the antennae separate, and then the legs one after another. The larva now falls forward on to its legs and pulls itself free. Hatching of eggs was observed throughout the day, but most hatching was noticed in the forenoon. The time required from the emergence of head to complete separation is usually 20–30 min.

(iv) *Habits of adult and larval stages.*

The adult thrips on coming out of hibernation feed first on the meadow grass and then migrate to young wheat seedlings and feed at the leaf-bases, inside the leaf-sheaths or in the folds of the growing leaves. Here they oviposit. When the earlier leaves become tougher they move to newer tender leaves and feed and oviposit there. Later on when the ears are being formed and are still enclosed inside the last folding leaves, they feed on the glumes in the tender stage. As the ears grow out they go inside the glumes and oviposit there. The females when feeding seeks out a tender place. After finding one, it applies its mouth-cone and a thicker rod (probably the mandible) is first inserted in the tissue and the mouth-cone is slightly jerked and then the thinner rods (maxillary stylets) work their way in. The mouth-cone is now applied closely and the further process is not visible, but probably consists of sucking the fluid. The insect moves from place to place and repeats the operation. The sucking, being repeated at short intervals, causes the formation of white spots or streaks.

The larvae hatching from early laid eggs migrate to sheltered places inside the leaf-sheaths or folds of leaves and join the adults in feeding. The larvae hatching, before the ears are out, feed on the young developing ears. Later on, the larvae go inside the calyces or corollas of the flower. Larvae, hatching from eggs laid later in the calyces, join the older larvae from the leaf-sheaths and the adults; and all feed simultaneously in ears.

Pre-pupae and pupae of males and females, which are found inside the leaf-sheaths or florets, do not feed and are mere resting stages.

(v) *Moulting.*

There are four moults from egg to adult, giving two larval stages and two non-feeding pupal stages, the first pupal stage being of short duration.

The exuviae of the first and second stage larvae are thicker than the pre-pupal and pupal skins which are thin and translucent. The chitinous

coverings of antennae, legs and internal mouth spines are visible in the larval casts and are darker in the case of second stage larvae. The process of moulting is as follows: the first stage larva does not feed for a day or two before moulting, as the intestine does not appear to contain any green material. The larva becomes sluggish and motionless. The colour is pale yellow. The antennae are brought close together and the head is bent downwards, arching the body slightly. The tail part is first observed to empty. The legs are also tucked in. The skin at the top of the head bursts, and the larva slowly wriggles out separating the legs from the old skin one after another. The contraction of the tail-end and the legs, inside the old skin, appears to exert a force on the anterior region of the head where the skin bursts longitudinally. The process of moulting in the second stage larva, pre-pupal and pupal stages is similar. The time required for moulting in the larval stages was found to be 12-20 min. and in the pupal stage as much as 30 min. or more.

(vi) *Life history.*

Eggs are laid from early June onwards. The following records, out of many, are selected, four from each month, to give the life cycle of the eggs laid in June, July and early August, and also the duration of larval, pre-pupal, and pupal stages under laboratory conditions.

Date of oviposition	Date of pupal moult	Egg	Length of stages in days				Total life cycle in days
			1st larva	2nd larva	Pre-pupa	Pupa	
5. vi. 30	9. vii. 30	11	6	8	2	7 ♀	34
6. vi. 30	5. vii. 30	10	5	7	1	6 ♀	29
29. vi. 30	1. viii. 30	11	5	9	2	6 ♀	33
30. vi. 30	3. viii. 30	13	6	8	2	5 ♀	34
3. vii. 30	5. viii. 30	12	4	10	3	4 ♀	33
11. vii. 30	9. viii. 30	13	5	5	2	4 ♀	29
11. vii. 30	15. viii. 30	12	6	9	2	6 ♀	35
22. vii. 30	23. viii. 30	10	5	7	3	7 ♀	32
1. viii. 30	3. ix. 30	12	6	8	2	5 ♀	33
1. viii. 30	31. viii. 30	11	6	8	2	3 ♀	30
2. viii. 30	2. ix. 30	10	7	9	1	4 ♀	31
3. viii. 30	6. ix. 30	11	7	8	2	6 ♀	34

It will be seen from the above that there is no appreciable lengthening of the life cycle during July and August. The slight differences in days appear to be due to individual differences. The egg period is from 10 to 13 days, the first larval period from 5 to 7 days, but once one first-stage larva moulted in 4 days. The second larval period is from 8 to 10 days usually, but once one second-stage larva moulted in 5 days. The pre-pupal period usually lasts 2-3 days, but sometimes moulting occurred after 1 day. The pupal period is from 6 to 7 days generally, but occasionally

moulting occurred on the fifth or even fourth day and once one male pupa moulted after 3 days. This appears to be unusual, but the number of male pupae observed was not large. The total life cycle from an egg to the adult requires 30 to 35 days, but twice the period required for an adult to emerge was 29 days. The emergence of the last adult in the laboratory was on September 6th.

(vii) *Flight.*

Flight was observed several times on bright sunny days in wheat and oat fields when males and females come out and wander over the ears of corn, some of them pairing and some of them going towards the top of the ears. The females, after reaching the top of the awns, turn round backwards and throw their front legs in the air. They then turn their tails upwards several times, separating the wings each time, which extend nearly at right angles to the body but close up when the tail is turned down. At one of these moments of extending the wings, the thrips fly off. Occasionally they jump only to another ear. As long as the sun is bright a large number of thrips are observed flying. The distance covered by a single flight was found to be 5-8 ft. in the still air of the laboratory. It appears that in the field they must be carried long distances over the grass and other places through the agency of winds.

(viii) *Mating.*

Mating can be observed in the field on ears when males and females congregate on sunny days. In the laboratory mating was observed inside a calyx where a male and a female were enclosed. The male at first does not seem to be able to see the female even from a short distance. They keep on feeding and accidentally meet each other. On one of these moments the male seems excited as the copulatory organ is seen projecting and retracting. The male now climbs over the female's back, turns its abdomen underneath, and pairing takes place. This position is similar to that described by Buffa (1907). There are short abdominal contractions and after that the male remains motionless for about 6 min. and then separates. A male is able to fertilise several females.

(ix) *Proportion of sexes.*

The first collection of thrips (May 21st) from meadow grass consisted of females only, and only females were found on wheat and oats till late June. Males were first found on June 29th. These were of the new generation. During July, August and early September males were found,

sometimes in enormous numbers. The following counts from oats and wheat give the proportion of the two sexes collected:

Date	Locality	Food plant	Males	Females
July 23rd	Liberton	Oats	433	162
" 24th	"	Wheat	257	351
" 28th	"	"	61	28
Aug. 2nd	"	" (1 head)	4	15
" 5th	"	" (3 heads)	7	30
" 23rd	"	"	1	10

Wheat was harvested on August 25th and the following collections were made from bundles in field:

Date	Locality	Food plant	Males	Females
Aug. 25th	Liberton	Wheat	5	25
Sept. 3rd	"	Oats	112	51
End of Sept.	"	"	None	A few
Aug. 29th	Boghall	Wheat	6	21
" "	"	Oats	5	10
Sept. 15th	"	"	0	6

Only females were found in late September and October. Males are apterous and appear to die after fertilising the females. In the laboratory males were found till October 14th and after that only females were visible.

(x) *Food plants.*

This species feeds mainly on Gramineae, and has been collected from various meadow grasses, wheat and oats. In Denmark (Ferdinandson and Rostrup, 1921) and Norway (Schøyen, 1924) it has been found on barley, and in Bulgaria (Chorbadzhev, 1929) on rye. Occasionally it has been taken from blackberry (Theobald, 1926) in England. In the Edinburgh district it was taken from wheat, oats and meadow grasses.

(xi) *Nature and extent of damage to crops.*

(a) *Wheat.* Heavy oviposition causes blotching of leaves. The feeding of larvae and adults removes chlorophyll, and this causes the appearance of white patches. The adults and the larvae, that feed on the tender parts of the growing ears both from above and below during the period the ears are confined inside the leaf-sheaths, cause so much damage in proportion to infestation that the individual florets fail to develop seeds. The longer the ears remain inside the folding leaves the greater the chances of damage.

Jablonowski (1926) considers that the importance of thrips has been overestimated and that they are not responsible for the damage that is constantly ascribed to them. According to him the injury is due to mechanical causes, chiefly winds. According to Körting (1930) the

cause of "white ear" disease in rye, oats or barley is not due to *L. cerealium*. In order to determine the nature and extent of injury to wheat plants, a set of experiments were carried out in the laboratory. Two sets of plants were grown in as similar conditions as possible. One lot were heavily infested with thrips and the other lot kept free. Plate XXI, fig. 1, shows the ripe ears: *A-B* from infested plants and *C-D* from free plants. It is seen in ears *A-B* that the lower and top parts have failed to develop seeds and the intermediate parts are also damaged. In uninfested plants the ears *C-D* are long and stout and have fully developed seeds at the base and top. In the fields, however, such contrasting differences are rarely seen owing to the other factors that keep a check on the abnormal damage to the ears.

An actual extent of damage to wheat was established by taking ears of wheat at random from Liberton and Boghall fields. The number of adult and immature thrips was also counted.

A. Liberton wheat fields.

Date	No. of ears taken	Total no. of florets	No. unset	% damaged	No. adults	No. young
24. viii. 30	50	1163	209	17.9	608	576

B. Boghall expt. farm fields.

8. viii. 30	10	208	4	1.9	12	11
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From the above figures it will be seen that at Liberton the damage was much greater than at Boghall. There was also a greater infestation of thrips at Liberton. The highest number of thrips found in a head was 125 adults and 25 larvae.

(b) *Oats*. The injury to oats is of a similar nature but much less evident in the leaves. The inflorescence is attacked before it leaves the leaf-sheath, and in proportion to the number of adults and larvae present, the basal part is subject to injury for a longer time. The flowers at the base appear as thin, white, shrivelled spikelets. Plate XXI, fig. 2, shows the spikelets at the basal part of the inflorescence damaged by thrips.

The extent of injury to oats was calculated by counting the unset seeds in the affected inflorescence:

Date	No. of damaged info.	Total no. of spikelets	No. unset	% damaged	Adult thrips	Immature forms
23. vii. 30	71	1611	618	38.3	595	2976

From the above figures it will be apparent that the percentage of unset spikelets in affected inflorescence is very high.

(xii) *Hibernation.*

The adult female thrips start migrating from the grown crop to the grass before the crop is harvested and continue after that from the corn bundles lying in the fields. The insect hibernates in grass during autumn and winter. Thrips have been collected in late August and September by pulling out grass from the fields where females were found hiding in the folds.

(xiii) *Seasonal history.*

L. cerealium were first collected over meadow grass on May 21st, 1930. On subsequent dates the number of thrips in each collection from meadow grass increased, till by May 26th large numbers were found. These later on migrated from meadow grass to wheat, and by June 4th or 5th all the wheat plants were severely infested. Males and females of the new generation were observed on wheat for the first time on June 29th in the field. By July 27th the number of immature stages decreased and more adult males and females were found. In August no first-stage larvae were found in fields. Only pre-pupae and pupae were found. The crop was harvested on August 25th. After that only adult females were found inside the grass leaf-sheaths.

This species appears to be single brooded here. Females oviposit and feed during summer on wheat and oats and finally migrate to grass or other places for shelter and hibernation. As the early females keep on laying eggs for considerable time all the stages are found simultaneously and there is an overlapping of early females with the females of the new generation.

IV. *APTINOTHRIPS RUFUS* GMELIN.(i) *Egg laying and hatching.*

Eggs are found in the form with six-jointed antennae all the year round, but in the variety *stylifera* (with eight-jointed antennae) eggs have been noted only in summer. The eggs are laid in slits, as in *L. cerealium*, and are scattered all over the leaf surface.

The eggs, when mature, appear dirty brown with two small red spots and the larvae come out by slow forward and backward movement as in *L. cerealium*.

(ii) *Food plants.*

This species lives mainly on various grasses and can be collected at any time of the year. In the summer it has been found also on oats, wheat and barley. Sometimes it is found in considerable numbers on young wheat seedlings, inside the leaf-sheaths, near the roots.

(iii) *Life history.*

An egg laid on July 31st hatched on August 18th and two eggs laid on September 2nd hatched on September 21st and 22nd respectively. This gives the egg period of 18-20 days in August and September. The first-stage larva hatching on August 18th moulted on August 26th, and the second larval moult occurred on September 1st. This gives the first larval period of 8 days and the second larval period of 6 days. The third moult occurred on September 3rd and the adult female emerged on September 9th. This gives the pre-pupal and pupal periods of 2 and 6 days. The whole life history from the egg laid on July 31st required 40 days. This species is many-brooded and the broods overlap.

(iv) *Seasonal occurrence of the two forms and males.*

The six- and eight-jointed forms are found throughout the year on grasses. The six-jointed form has frequently been found to occur alone. At certain localities both forms are found in the same collection. It has been observed sometimes that the two forms may occur separately even within a few yards of one another. At certain localities only eight-jointed forms occur. This isolated distribution of the two forms is very remarkable. Williams (1913) also had a similar experience with this species in the south of England. He observed that the commonest form was six-jointed, while the eight-jointed was scarcer and more local. The two forms did not usually occur together, and out of many records he only twice got both forms together. Morison (1924), during collections of thrips between October 23rd, 1923, and January 27th, 1924, from Aberdeen area, found that females of *A. rufus* taken by beating grass at three different localities (near Bridge of Dee Fishery Station, Craibstone Experimental Farm) were each time associated with *A. rufus* var. *conaticornis* Uzel (six-jointed form). He also found that the six-jointed form was very common. The summarised results of the collections made in 1930 with the locality and the forms collected therefrom are given in the table on p. 319. All the localities are in the suburbs of Edinburgh.

It will be seen from these that at King's Park (area A) only the six-jointed form was found all the year round. From area B only two collections were made in September and October and only the eight-jointed form was found. At area C the two forms were found mixed up in different proportions. It is possible, however, that they were really separate, as the area of collection was slightly larger. From the Blackford Hill area C the two forms appeared mixed up in different proportions, and once from area A 138 were collected all six-jointed. From the area

B all the thrips were eight-jointed. From the Morningside locality no eight-jointed form was found at area A, and at area B all the thrips collected were eight-jointed. At area C the six- and eight-jointed forms were found mixed up in different proportions. Unfortunately the collections at area B were made only for 3 months as the grass was later on cut and removed.

Month	King's Park areas*			Blackford Hill areas†		Morningside areas‡		
	A		C	C		B	C	
	Mixed		Mixed	Mixed		8 j only	Mixed	
	6 j only	8 j		6 j	8 j		6 j	8 j
November	579	16	10	—	—	85	112	104
December	292	54	45	—	—	100	172	98
January	115	31	16	—	—	212	—	—
February	71	—	—	209	139	Grass cut and removed		
March	52	—	—	170	131	—	—	—
April	42	22	2	21	42	—	—	—
May	88	10	29	—	—	—	—	—
June	107	59	22	—	—	—	—	—
July	120	—	—	270	44	—	—	—
August	57	—	—	78	99	—	—	—
September	117	—	—	—	—	—	—	—
October	126	—	—	—	—	—	—	—
Total	1766	192	124	748	455	397	284	202

Note. 6 j=six-jointed form; 8 j= eight-jointed form.

* At King's Park, area B, only 28 eight-jointed females were taken in September and October each.

† At Blackford Hill, area A, only 138 six-jointed females were taken in April. At area B, 45 eight-jointed females were taken in August.

‡ At Morningside, area A, none was taken.

Larvae have been found all the year round only from such localities where the six-jointed form occurs. The number of larvae found was greater in winter, less in spring and least in autumn. The number of larvae, pre-pupae, pupae, males and females collected during the year is summarised in Table I.

It will be seen from this table that one pre-pupa was found in January, four in April, and one in May and June each. Pre-pupae usually remain at sheltered places and beating of grass, unless heavy, does not bring them out easily. In all seven pre-pupae were captured throughout the year.

Except in November 1929 and in February, March and October 1930, pupae were found throughout the year in different proportions. In all thirty-eight pupae were captured.

Males are very scarce in this species. Uzel (1895) in Bohemia and Hinds (1902) in America did not find any males. Bagnall (1910) and

Table I.
The number of six- and eight-jointed forms with eggs; and the number of males, larvae, pre-pupae and pupae collected in 1930.

1929-30 Month (1929)	Six-jointed females						Eight-jointed females						Males and young stages of six-jointed form							
	No. of eggs						No. of eggs						No. No. No. pre- No. males larvae pupae pupae							
	No.	1 egg	2	3	4	5	Total	%	No.	1 egg	2	3	4	5	Total	%	No.	No. No. pre- No. males larvae pupae pupae		
Nov.	697	—	1	—	—	—	1	0.14	209	—	—	—	—	—	—	—	1	50	—	—
Dec.	515	14	7	2	1	—	24	4.6	153	—	—	—	—	—	—	—	—	102	—	2
(1930)																				
Jan.	156	42	7	—	—	—	49	31.4	16	—	—	—	—	—	—	—	—	44	1	2
Feb.	280	15	25	1	1	1	43	15.3	139	—	—	—	—	—	—	—	3	106	—	—
March	213	21	16	—	—	—	37	17.3	131	—	—	—	—	—	—	—	—	49	—	—
April	302	126	42	0	—	—	174	57.6	48	39	6	—	—	—	45	93.7	2	43	4	17
May	82	35	13	7	1	—	56	68.2	54	16	29	1	1	1	48	88.8	—	22	1	8
June	189	70	20	10	1	—	101	53.4	30	11	5	1	—	—	17	56.6	5	30	1	4
July	270	48	19	4	—	—	71	26.2	44	—	—	—	—	—	—	—	3	20	—	1
August	135	8	6	2	2	—	18	13.4	45	—	—	—	—	—	—	—	—	19	—	2
Sept.	117	13	15	1	—	—	29	24.7	18	—	—	—	—	—	—	—	1	3	—	1
Oct.	126	10	11	—	—	—	21	16.6	28	—	—	—	—	—	—	—	—	6	—	—
Nov.	130	6	1	—	—	—	7	5.3	10	—	—	—	—	—	—	—	—	1	—	1
															Total		15	495	7	38

Williams (1913) also record the rarity of males. Williams in September 1913 found only twenty males among many hundred females. Morison (1924) during his collections between October 23rd, 1923, and January 27th, 1924, from the Aberdeen area, collected 200 females, 10 males, 35 larvae and 4 pupae. This gives a fairly large proportion of males. In England he found one male in winter among several hundred females. I found only fifteen males out of several hundred females in the whole year. All the males collected were six-jointed and no male with eight-jointed antennae was seen. Recently Morison has sent me a slide of a male of the eight-jointed form collected by him on August 18th, 1928, in Aberdeen.

The greater preponderance of the six-jointed females and the rarity of males restricted to certain small areas and the wider and commoner distribution of females only, suggest that over large areas the six-jointed form is purely parthenogenetic. At those places where males occur, a limited percentage may be sexual, as even there the females are in the greater majority. The extreme rarity of the males in the eight-jointed form is suggestive that over a greater area it is apparently parthenogenetic like the six-jointed form; and that it is only sexual in very limited localities.

(v) *Seasonal and relative fecundity in the two forms.*

From the first collection made in November 1929 the six-jointed form has been found to contain eggs in various proportions all the year round.

As seen from Table I, few females contained eggs in November, but in succeeding months the number of females with eggs increased. In spring and summer a very great number of females were found to contain eggs. In May the percentage of females containing eggs was highest for the year. From July onwards the number of females with eggs decreased so much so that again in November 1930 the number of females with eggs was lowest.

Individuals with one or two eggs were very common, but sometimes as many as five eggs were visible in one female.

The eight-jointed form which occurs usually separately or occasionally mixed with the six-jointed form was not found to contain eggs till March in any of the collections. Eggs were first noted in them on April 23rd, 1930, from a collection made at Balerno. In this collection about 93.7 per cent. of females contained eggs. During May and June a considerable number of females were found to contain eggs. From July onwards no

eggs were noted. In this form also, during breeding season, individuals with one or two eggs were very common, and in May as many as five eggs have been found in a single female. It appears probable that breeding occurs for a short term of the year in this form.

Table I also gives the summarised figures of the fecundity of the two forms and the number of females with number of eggs from November 1929 to November 1930.

This difference in the rhythm of the breeding periods of the six- and eight-jointed forms, if universal, presents some interesting questions of specific importance. Recapitulating what has been said before, it appears that the eight-jointed is a definite form with a life cycle of its own, breeding for a short period, usually living isolated and having fewer broods in a year. The six-jointed has been considered as a variety of eight-jointed form by Uzel, Bagnall and others. But, seeing that it differs in the number of antennal joints, usually lives separately and breeds all the year round with a considerable number of broods per year, it seems desirable that structural difference, correlated with biological difference, should be sufficient to separate this form into a distinct species. However, there are certain other points which are also to be taken into consideration.

Recently Radulesco (1930), experimenting at Saint-Genis-Laval (Rhône), France, considers that the eight-jointed form under certain conditions of temperature and dryness gives rise to the six-jointed form. Priesner (1928), during some of the collections, found a form with seven-jointed antennae (f. *intermedia* Pr.), and from a large collection of the eight-jointed form I got some females having one normal six-jointed antenna and the other normal eight-jointed. These various records seem to lend support to the view that the eight-jointed form may under certain conditions give rise to the six-jointed form.

The collection of an eight-jointed male by Morison in Aberdeen opens up the field for supposing that the eight-jointed form has also a small sexual race. It appears to me, therefore, that there are four races, the sexual and parthenogenetic six-jointed race and the sexual and parthogenetic eight-jointed race. Since these two forms are so closely allied, the eight-jointed form under certain conditions may produce individuals of the type of seven-jointed antennae (forma *intermedia* Pr., p. 158, 1928), and the six- and eight-jointed antenna in the same individual noticed by me (1932*b*).

V. NATURAL ENEMIES.

1. *L. ceralium*. No parasite of this species was found. The carnivorous *Aelothrips fasciatus* reported to feed on thrips was never found attacking this species.



Text-fig. 1. A predaceous *Trombidium* larva attached near the hind coxa of *Aptinothrips rufus* Gmelin.

2. *Aptinothrips rufus*. One predaceous *Trombidium* mite was found attached near the base of hind coxa on one or the other side of the insect. These *Trombidium* larvae appear to suck the fluid out of the abdomen, but otherwise do not seem to affect the thrips, as some of them contained mature eggs. (Text-fig. 1.)

An internal nematode parasite was found at certain localities in Edinburgh. The infested thrips generally become sterile and in some

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cases die. The biology of the nematode parasite is discussed in a separate paper (1932*a*).

VI. SUMMARY.

1. The insect *L. cerealium* feeds on wheat and oats and on various meadow grasses in spring and summer.

2. The method of egg-laying, hatching, and the habits of adult and immature stages are noted and described.

3. The total life history from the egg to adult takes 29–35 days; egg period 10–13 days, larval period 13–17 days, pre-pupal period 2–3 days and pupal period 6–7 days.

4. *L. cerealium* has been found to be responsible for non-setting of seeds in wheat and oats. The character of the injury is described. Laboratory experiments on wheat show that in proportion to infestation the injury to ears is increased.

5. Males have short life. The fertilised females fly off, after the crop is harvested, and hibernate in grass or other sheltered places during winter. The emergence of adult females from hibernation was about late May in 1930. This species appears to be single brooded here.

6. In *Aptinotherips rufus* the method of egg-laying, hatching, etc., is similar to *L. cerealium*.

7. The two forms of this species differing in the number of antennal joints are generally found separate but occasionally mixed up in various proportions. The distribution of the two forms and the ratio collected from various localities are given with number of males, females, and immature stages.

8. The six-jointed form is found to contain eggs all through the year and the eight-jointed form only for a few months of the year. The relationship of the two forms is discussed and it is suggested that there appears to be four races within the species, a sexual and a parthenogenetic race of each form.

9. An internal nematode parasite was found in *Aptinotherips rufus*. The parasite causes sterility in almost all cases. Predaceous *Trombidium* larvae were also found attached to *A. rufus*.

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EXPLANATION OF PLATE XXI.

Fig. 1. Ears of wheat from infested and free plants. *A, B* are damaged ears from infested plants. *C, D* are healthy ears from free plants.

Fig. 2. Sterile spikelets in oats showing injury by *L. cereálum*.

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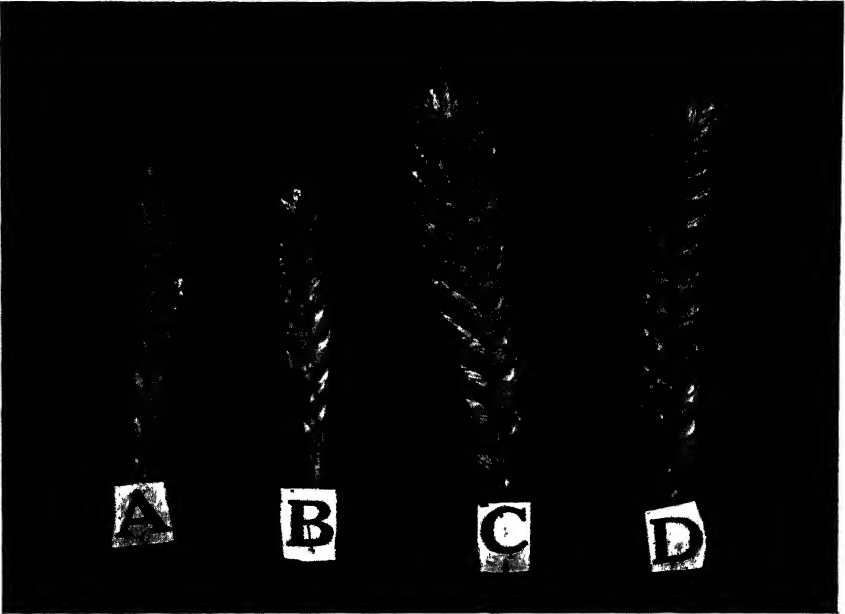


Fig. 1.

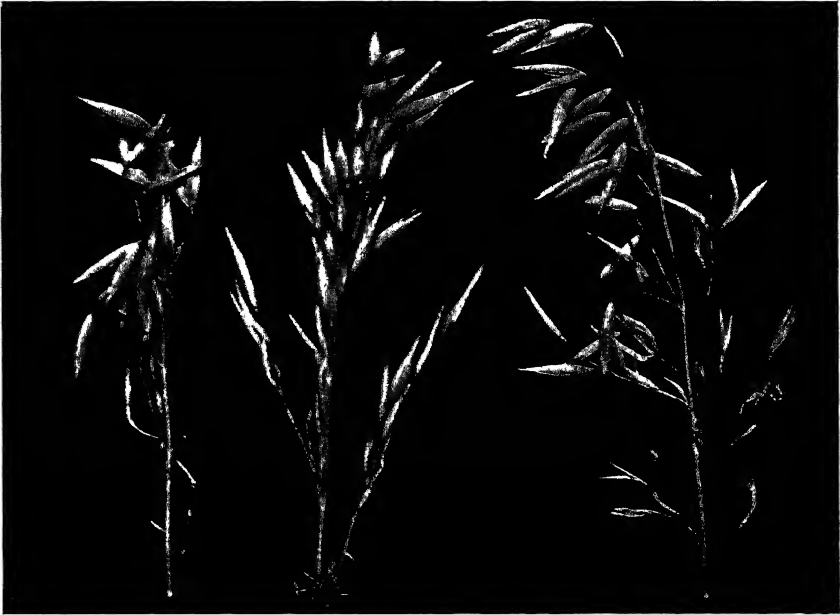


Fig. 2.

SOME CECIDOMYIDAE ATTACKING THE SEED OF *DACTYLIS GLOMERATA* L. AND *LOLIUM* *PERENNE* L.

BY MARGOT E. METCALFE, PH.D.,

Fellow of the University of Wales.

(Entomology Department, Rothamsted Experimental Station.)

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I. INTRODUCTION.

It has long been known that Cecidomyid larvae are responsible for damaging the grain in cereals. Similarly grasses grown for seed are subject to their attack. Information on this subject has recently been collected from scattered literature by Tomaszewski (1931) who deals with European species attacking stems as well as seed, and Barnes (1931) whose list includes species recorded from the seed only, but from all parts of the world. Three species are included by Tomaszewski which do not appear in Barnes' paper, viz. *Dasyneura airae* Kieffer on *Aira flexuosa*, *Cecidomyia poae* P. de B. on *Poa trivialis* and the larva of an unnamed species on *Phleum pratense* which Barnes refers to as only found in England.

Besides giving this list of midges already recorded Tomaszewski reported that he found Cecidomyiidae attacking the seed of the following grasses on the meadows at Randowbruch [Germany]: *Aira caespitosa* L., *Calamagrostis arundinacea* Roth, *C. epigeios* L., *C. neglecta* Fr., *Phalaris arundinacea* L., *Poa pratensis* L., and *P. serotina* Ehrh. He identified the midge on the *Poa* spp. as a species of *Contarinia*, but was unable to decide for himself whether or not it was a distinct species, as it was structurally

very similar to *C. tritici* (Kirby) and *C. merceri* Barnes. The damage at Randowbruch was reported by von Oettingen (1929) to be 60–75 per cent. in a normal year, and in several years 90 per cent. of the grass seed was lost. Tomaszewski states that the loss in 1929 and 1930 was 75–80 per cent. of the crop. The damage to seed of *Alopecurus pratensis* by Cecidomyids in England has been so severe that in many cases its use has been discontinued in favour of Timothy (*Phleum pratense*) (Barnes, 1930).

It was decided to investigate whether and, if so, to what extent, Cocksfoot (*Dactylis glomerata*) and Perennial Ryegrass (*Lolium perenne*) were subject to such attacks in Great Britain. Previously to this study *Contarinia dactylidis* (H. Lw.) had been recorded from the flower heads of *D. glomerata* in Germany, and Bagnall and Harrison (1918) also had recorded Cecidomyid larvae in *D. glomerata* spikelets in Durham, England.

The three midges of which an account is given in this paper have only been under observation for a year (June 1931 to July 1932), but the main features of the life histories have been worked out in each case. The observations were made at Harpenden which is about twenty-five miles north of London.

II. METHODS.

During the first weeks of June 1931 daily inspections were made of the grasses on the Park Grass Plots at Rothamsted. Female midges were observed ovipositing on heads of *D. glomerata*, *L. perenne*, *Bromus* sp., and *Avena* sp. It was decided to study those on the first two grasses. On June 15th larvae were found, and on June 27th samples of infested heads were collected. These were placed on damp coconut fibre, protected by a lamp glass covered by muslin-covered iron rings¹. The midges emerging the following spring were used for estimating the extent of infestation and biological studies, including immunity experiments. For these, plants of *Alopecurus pratensis*, *Dactylis glomerata*, *L. perenne*, *F. rubra* var. *arenaria* were obtained and planted out of doors at the laboratory. Other grasses were also planted but did not flourish and so were not used. The plants selected for experiments were covered with muslin cages before the flowers opened, and the midges were later introduced into them. From time to time the heads were examined for eggs and for larvae in the different instars. About three weeks after the plants had been infested all the heads were cut and brought indoors. Here they were sprayed with water at intervals, and the larvae which came out were placed after examination on damp fibre in order to observe pupation. Cross-mating

¹ See Imms, A. D., Some methods of technique applicable to entomology, *Bull. Ent. Res.* (1929), xx, 170.

experiments on the adults were also attempted. Owing to the limited period available no attempt has been made to discover the distribution of the midges described.

III CECIDOMYIDAE ATTACKING *DACTYLIS GLOMERATA* L.

Two Cecidomyids are responsible for the destruction of seed in Cocksfoot—they are *Dasyneura dactylidis* sp.n. and *Contarinia dactylidis* (H. Lw.).

1. *DASYNEURA DACTYLIDIS* SP.N.

(a) Identification.

No *Dasyneura* has hitherto been recorded on *D. glomerata*, but three species of *Dasyneura* have been found damaging grass seed, viz. *D. airae* Kieffer, *D. alopecuri* (Reuter) and *D. graminis* Felt. The original descriptions of these species differ from *D. dactylidis* in the following particulars:

D. airae. "Antennes de 2 + 12 articles. Les deux premiers articles du funicle sont soudés ensemble; chez le mâle ils sont 1 fois $\frac{1}{2}$ aussi longs que gros, les suivants une fois et quart, le dernier un peu plus long que les précédents, largement arrondi au bout; le col du premier article n'atteint que la moitié de la longueur de ce dernier, celui du second environ les trois quarts, celui des suivants atteint presque la longueur de l'article. Parfois l'antenne se compose de 2 + 13 articles; dans ce cas le dernier article est sensiblement plus court que les précédents..."

D. alopecuri. "Taster...; erstes Glied kurz, mehr breit als lang, abgestutzt; zweites etwas $1\frac{1}{2}$ so lang als dick, drittes ein wenig länger als zweites, über die Mitte hin schwach verdickt, viertes am schlanksten und zugleich am längsten, beinahe $1\frac{1}{2}$ mal so lang wie das drittes, allmählich verjüngt, etwas spitzig...die Länge der fünf Tarsenglieder verhält sich wie 1 : $5\frac{1}{2}$: 3 : 2 : $1\frac{1}{2}$...untere Lamelle ein wenig länger, lineal, am Ende ausgerandet..."

D. graminis. "Female. Length 1.5 mm. Antennae:...13 subsessile segments; terminal segment greatly produced, apparently composed of two closely fused segments, broadly rounded apically. Palpi; first segment short, stout, sub-quadrate, slightly swollen basally, the second a little longer, stouter, the third one-half longer than the second, more slender, the fourth one-half longer than the third."

I have also examined specimens of both the latter species in the Barnes collection and am satisfied that they are different species from the one on *D. glomerata* which is described below.

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Male. Body length: 1.5–2 mm. Antennae: 2 + 16; fuscous brown; of typical *Dasyneura* structure; length of neck of third flagellar segment two-thirds that of node, three and two-fifths times as long as broad; neck of fifth flagellar segment equal in length to node, fourth one and a half times as long as broad; flagellar segments with a distal regular whorl of setae reaching to about one-half the length of the succeeding node, and with irregular scattered setae about three times the length of the node. Face: yellow. Eyes: black. Palps: pale yellow; basal segment quadrate, second twice the length of the first, third equal in length to the second, fourth segment about two-thirds as long as the second and third together; basal segment as long as broad, second two and a half times, third three times, fourth segment five times as long as broad; sparsely setose. Thorax: dorsal region dark fuscous. Wings: hyaline; wing-veins scaled. Legs: pale yellow; clothed with dark fuscous scales. Claws: bifid; moderately curved. Empodium: rather longer than the claws. Abdomen: reddish with dorsal bands of dark fuscous scales. Genitalia: basal clasp segment stout, not swollen, with well-developed setae; terminal clasp segment stout, slightly curved; dorsal plate deeply bilobed, the lobes triangular with well-developed setae; ventral plate bilobed; harpes well developed, irregularly digitiform; style stout, longer than the ventral plate. Cotypes: Cecid. 1873–6 inclusive, deposited in the Barnes Collection.

Female. Body length: rather longer than in the male. Antennae: 2 + 12 to 2 + 14, usually 2 + 14; fuscous brown; of typical *Dasyneura* structure; neck of third flagellar segment transverse, about one-twentieth the length of the node; nodes with two regular whorls and irregular scattered setae. Ovipositor: pocket type, about twice the length of the body when extended. Otherwise similar to the male. Cotypes: Cecid. 1877–80 inclusive, deposited in the Barnes Collection.

(b) *Life history.*

On June 1st, 1931, the midges were first observed ovipositing in the field. The larvae of this brood were full grown and ready to drop to the soil for pupation by June 29th, though the last larvae did not leave these heads until August 4th. Here again, as in *D. leguminicola*, the clover seed midge, the period spent by the fourth instar larvae in the grass heads may be prolonged by about four weeks after the larva is full grown.

The midge is single-brooded in Harpenden, and in 1932 emergence took place between May 21st and June 6th with the crest of emergence on May 29th. The males appear earlier in the season than the females, the

greatest numbers of males being obtained on May 23rd and of females on May 24th. Out of 304 emergences there were 107 males and 197 females, giving a ratio of 35 males to 65 females.

Mating takes place very soon after emergence, and the females appear ready to lay their eggs immediately. Females placed on plants at about 3 p.m. (standard time) on May 30th started ovipositing immediately and egg laying was continued on the following day. The ovipositor appears to be thrust down between the paleae. Unfortunately no eggs could be found, neither were any first or second instar larvae obtained. On June 23rd, however, larvae in what appeared to be the third instar were found. These had an average measurement of 1.452×0.488 mm.; they were pale pink in colour, with the anchor process fully developed and pale yellow.

All the infested heads were brought into the laboratory on June 27th and sprayed with water: a very few full-grown larvae came out of the heads, and these, when placed on coconut fibre, went down for pupation.

This failure to raise the midge in any numbers may have been due either to the state of development of the grass heads, which after the cold and wet May were as yet unopened, or to the presence of thrips within the flowers. These insects have been said to feed upon the eggs of the clover flower midge (Pergande, 1882), and as they were present in the Cocksfoot heads in large numbers they may have been responsible for the destruction of many eggs.

(c) *Damage.*

The damage is done by the larva which eats away the ovary. The larvae feed singly, one larva destroying one seed, and the following tables show the infestation figures on Park Grass Plots in 1931:

Sample no.	No. of grass heads	No. of larvae	Average larvae per head
1	30	71	2.4
2	30	31	1.03
3	30	13	0.43
4	30	4	0.13
5	50	35	0.70
6	58	30	0.50
7	108	69	0.64
8	144	96	0.66

This gives an infestation of slightly less than one larva per head of grass.

In addition, samples, each of 30 heads of Cocksfoot, were placed on coconut fibre. The adults and parasites which emerged in 1932 give the following infestation:

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Sample (30 heads each)	♂♂	♀♀	Para- sites	Average parasitism	Total emergences	Average per head
CFA	9	15	20	45 %	44	1.46
CFB	10	15	18	42	43	1.43
CFC	11	10	31	60	52	1.73
CFD	5	3	16	66	24	0.80
CFE	3	4	16	66	23	0.76
CFF	29	37	14	17.5	80	2.66
CFG	12	38	56	53	106	3.53

Supposing each parasite destroys one midge larva, these figures would indicate that, on the average, slightly below two seeds in each head were destroyed.

An infestation of from 0.13 to 3.53 per head cannot be considered serious since a head of Cocksfoot may contain from 500 to 1500 flowers. The damage to Park Grass is therefore at present negligible, but the latent possibilities of such a pest cannot be ignored.

(d) *Parasitism.*

Parasitism of the 1931 brood ranged from 17.5 per cent. in Sample CFF to 66 per cent. in Sample CFE, with an average of 42.3 per cent. for the seven samples: this would not in itself constitute an effective control in a year of heavy infestation. The parasite is *Prosactogaster tisis* Walk.¹ [Scelionidae]. The parasites hatched between April 22nd and June 5th, with a crest of emergence on May 30th, one day after that of the midges.

2. *CONTARINIA DACTYLIDIS* (H. Lw.).

(a) *Identification.*

A Cecidomyid on *Dactylis glomerata* has already been recorded as *C. dactylidis* (H. Lw.). Loew's original description (1851) of this midge deals with the female only, and, as far as it goes, the *Contarinia* found on *D. glomerata* on the Park Grass Plots agrees with this description. Five other species of *Contarinia* are responsible for seed damage: *C. merceri* Barnes, *C. arrhenatheri* Kieffer, *C. avenae* Kieffer, *C. brizae* Kieffer, and an unnamed species on *Poa trivialis* described by Tomaszewski.

The following features distinguish these Cecids from *C. dactylidis*:

C. merceri. "Male. Body length 1-2 mm.;...the stem and neck of the third flagellar segment two or three times as long as broad, those of the tenth three to four times as long as broad...."

C. avenae. "Palpes de quatre articles qui sont, du premier au dernier, 1½, 2½, 3 et 5 fois aussi longs que gros...chez les femelles, les articles

¹ Kindly identified by Dr Ferrière of the Imperial Institute of Entomology.

inférieurs (des antennes) un peu rétrécis au milieu; le premier article est cinq fois aussi long que gros, et le col a le septième de sa longueur; le second et le troisième subégaux, deux fois et un tiers aussi longs que gros, avec un col qui atteint le tiers de leur longueur; les suivants deux fois aussi longs que gros, le col atteint la moitié de leur longueur, à l'exception des deux derniers articles, où il n'atteint plus que le tiers...."

C. arrhenatheri. "Femelle...les articles des palpes sont $1\frac{1}{2}$, 2, $3\frac{1}{2}$ et 4 fois aussi longs que gros...."

C. brizae. "...Rückenschild mit drei schwarzen Längstreifen. Hinterleib roth, mit dunkeln Querbinden...."

Contarinia sp. on *Poa trivialis*. "Weibchen...Gliederstiele ca. $\frac{1}{3}$ so lang wie die Geisselglieder. Längenverhältniss der Geisselglieder: I = 1.25; II = 0.85; III = 0.60; IV = 0.65; V = 0.60; VI = 0.70; VII = 0.65; VIII = 0.68; IX = 0.65; X = 0.70; XI = 0.70; XII (mit Griffel) = 1.0."

It is therefore considered that the species dealt with is identical with *C. dactylidis* (H. Lw.), an amplified description of which is given below:

Male. Body length 1-1.5 mm. Antennae: 2 + 12; fuscous brown; of typical *Contarinia* structure; basal and distal nodes of third flagellar segment about equal in size; stem and neck of third flagellar segment about equal in length and about three times as long as broad; stem about one and a half times as long as the basal node; stem and neck of tenth flagellar segment four to six times as long as broad; distal elongation of twelfth flagellar segment about half the length of the stem; each node with a whorl of six looped regular circumfila, arising distally and approximately equalling in length the stem or neck; each node also with a whorl of regular setae about one and a half times the length of the circumfila. Palps: pale yellow; sparsely setose; basal segment quadrate, second one and three-quarters times as long as the first, third two and a quarter times as long as the first, fourth segment about two and seven-eighths as long as the first; basal palpal segment about one and three-sevenths times as long as broad, second twice as long as broad, third three and two-fifths, and distal four and four-sevenths times as long as broad. Face: fuscous. Eyes: black. Thorax: fuscous brown dorsally, pleura yellow. Wings: hyaline. Legs: yellow with fuscous hairs. Abdomen: clear yellow. Empodium: equal in length to simple claws. Genitalia: basal clasp segment fuscous yellow, with long setae; distal clasp segment fuscous brown without setae; dorsal plate deeply bilobed, the lobes rounded; ventral plate deeply bilobed; style rounded, rather longer than ventral plate. Cecid. 1881-4 inclusive, deposited in the Barnes Collection.

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Female. Body length: rather longer than in the male. Antennae: 2 + 12; of typical *Contarinia* structure; first flagellar segment about one and a half times as long as the second, five times as long as broad, five times as long as the neck; third flagellar segment two and one-third times as long as broad, three and three-fifths times as long as the neck. Palps: basal segment quadrate, second one and one-fifth times, third one and one-seventh times, fourth segment two and three-tenths times the length of the basal segment; basal segment one and two-thirds times as long as broad, second one and a half, third two and five-sixths, fourth segment four and four-fifths times as long as broad. Wings: hyaline. Abdomen: bright yellow. Ovipositor: aciculate, very long and slender. Otherwise similar to the male. Cecid. 1895-8 inclusive, deposited in the Barnes Collection.

(b) *Life history.*

The midge was bred out in 1932 from the same heads of Cocksfoot as *Dasyniura dactylidis*. Emergence began on June 3rd, reached its maximum on June 11th and ended on June 24th, thus overlapping the brood of *Dasyniura* by four days. The males emerged rather earlier in the season than the females, but reached their crest of emergence on the same day, subsequently falling off in numbers very rapidly. The sex ratio was 34 : 66, 172 males and 339 females being reared.

Cocksfoot plants were infested with fertilised females on June 9th, 10th and 11th, but careful searching failed to reveal either eggs or young larvae. On July 1st all the heads were cut and brought indoors, where they were sprayed with water. A few larvae whose average measurements were 1.428×0.444 mm. left the heads. These, when placed on fibre, went down for pupation. The grass heads were again sprayed on July 6th when a few more larvae came out. The larvae, therefore, appear to be full grown, or at least ready for pupation, about three weeks after the eggs have been laid. In samples collected in 1931, larvae remained in the heads until August 4th. There thus appears to be a similar lengthening of the time spent as fully grown larvae in the grass heads, as occurs in *D. dactylidis*.

(c) *Damage and infestation.*

The damage is done by the larvae which feed collectively, five or six to the flower, on the ovary. The following numbers of larvae were obtained from samples taken from the Park Grass Plots in 1931. These samples were also used for the counts of *Dasyniura* larvae.

Sample no.	No. of grass heads	No. of Larvae	Average larvae per head
1	30	1	0.03
2	30	0	0.00
3	30	72	2.40
4	30	12	0.40
5	50	1146	22.90
6	58	1129	19.40
7	108	740	6.80
8	144	441	3.00

The infestation therefore ranged up to 22.9 larvae per head with an average of 6.86.

From the samples of 30 heads kept over winter the following midges and parasites were bred out in the spring of 1932:

Sample (30 heads each)	♂♂	♀♀	Parasites	Average parasitism	Total emergence	Average per head
CFA	31	60	22	19%	113	3.76
CFB	39	68	15	12	122	4.06
CFC	46	68	13	10	127	4.23
CFD	5	17	8	27	30	1.00
CFE	9	9	0	0	18	0.60
CFF	4	17	0	0	21	0.70
CFG	4	9	2	13	15	0.50

It will be observed that probably fewer larvae of this species came to maturity than in the case of *D. dactylidis*.

The average infestation in both larval and adult counts is very low, even where the larvae averaged 22.9 per head of grass. Since feeding is collective, probably not more than four or five ovaries would be destroyed.

(d) Parasitism.

Parasitism is generally lower than in *D. dactylidis*, and in the brood of 1932 the average was only 11.6 per cent. The crest of parasites coincided with the maximum numbers of midges emerging. The parasite again is *P. tisis* Walk.

(e) Immunity trials.

Attempts were made to infest *Festuca rubra* var. *arenaria* and *L. perenne* with *Contarinia dactylidis*.

Three weeks were allowed to elapse after the time of infestation, then the heads were cut, brought indoors and sprayed with water. This was repeated at intervals of five days, but no larvae were obtained. *F. rubra* var. *arenaria* and *L. perenne* may therefore be considered not subject to attack from *C. dactylidis*. This provides additional evidence to show that the *Contarinia* found on *L. perenne* is a distinct species. Cross-mating experiments were tried with these two midges, but with no success.

IV. CECIDOMYIDAE ATTACKING *LOLIUM PERENNE* L.

No Cecidomyid has previously been recorded from *L. perenne*, and the species herein noted is described as a new species of *Contarinia*, viz. *C. lolii*. It differs in the following features from the other *Contarinia* found damaging grass seed:

C. merceri. "♂ Circumfila in 7-9 regular loops. Stem and neck of third flagellar segment about two to three times as long as broad, those of tenth three to four times as long as broad."

C. avenae. "♂ Antennes: les quatre premiers cols sont un peu plus courts que leur nodosité; ceux des suivants un peu plus longs qu'elle; celui de la nodosité globuleuse, toujours plus long que celui de la nodosité ovulaire; le col de la nodosité terminale égale la moitié de la longueur de celle-ci."

"♀ Chez la femelle, le premier article est cinq fois aussi long que gros, et le col a le septième de sa longueur; le second et le troisième subégaux, deux fois et un tiers aussi longs que gros, avec un col qui atteint le tiers de leur longueur; les suivants deux fois aussi longs que gros, le col atteint la moitié de leur longueur, à l'exception des deux derniers articles, où il n'atteint plus que le tiers. Oeufs blancs."

C. arrhenatheri. "♀ les articles des palpes sont $1\frac{1}{2}$, 2, $3\frac{1}{2}$ et 4 fois aussi longs que gros."

C. brizae. "Hinterleib roth, mit dunkeln Querbinden."

Contarinia sp. on *Poa trivialis*. "♀ Gliederstiele ca. $\frac{1}{3}$ so lang wie die Geisselglieder. Längenverhältniss der Geisselglieder: I = 1.25; II = 0.85; III = 0.60; IV = 0.65; V = 0.60; VI = 0.70; VII = 0.65; VIII = 0.68; IX = 0.65; X = 0.70; XI = 0.70; XII (mit Griffel) = 1.0."

C. dactylidis. "♂ Palps: basal segment quadrate; second segment $1\frac{3}{4}$, third segment $2\frac{1}{4}$, fourth segment $2\frac{7}{8}$ times the basal segment."

The features distinguishing one species from another are clearly minor characters and cannot always be relied upon for the satisfactory separation of two species: the only reliable method at present is to attempt mating experiments. Thus while *C. dactylidis* differs from *C. lolii* only in proportion of the palp segments, the cross-mating experiment failed completely.

CONTARINIA LOLII SP.N.

(a) Description.

Male. Body length: 1-1.5 mm. Antennae: 2 + 12; fuscous brown; of typical *Contarinia* structure; nodes of third flagellar segment approximately equal in size; stem and neck approximately equal in length; stem

about one and one-third the length of basal node; stem about three to three and a half times as long as broad; stem and neck of tenth segment from four to six times as long as broad; distal prolongation of twelfth flagellar segment about five-sixths of stem; each node with an apical whorl of six regular, looped circumfila, those of basal node about equal in length to the stem, those of distal node about two-thirds the length of the neck. Palps: fuscous; second segment one and one-sixth as long as first, third one and two-thirds, fourth one and five-sixths as long as first; basal segment twice as long as broad, second one and three-fifths, third two and three-quarters, fourth segment five times as long as broad; sparsely setose. Face: fuscous. Eyes black. Thorax: fuscous yellow with a darker band dorsally. Wings: hyaline. Legs: yellow with fuscous scales. Claws: simple. Empodium: rather shorter than the claws. Abdomen: clear yellow. Genitalia: basal clasp segment swollen, with long setae; distal clasp segment fuscous; dorsal plate bilobed, the lobes rounded; ventral plate deeply bilobed, the lobes triangular, rounded apically; style about the same length as the ventral plate. Cotypes: Cecid. 1893-6 inclusive, deposited in the Barnes Collection.

Female. Body length: rather longer than in the male. Antennae: 2 + 12; of typical *Contarinia* structure; first flagellar segment one and two-thirds as long as second, five times as long as broad; node of third flagellar segment twice as long as broad, two and three-fifths as long as stem. Palps: basal segment twice as long as broad, second one and three-fifths, third three times, fourth three and three-quarters times as long as broad; second segment one and a half times as long as first, third one and seven-eighths times, fourth two and a half times as long as first. Abdomen: bright yellow. Ovipositor: aciculate, very long and slender. Otherwise similar to the male. Cotypes: Cecid. 1889-92 inclusive, deposited in the Barnes Collection.

(b) *Life history.*

C. lolii is single-brooded at Harpenden, the adult midges being on the wing in June, and the larvae overwintering in the soil. In 1932 the first midges appeared on May 31st and the last on June 26th, the crest of emergence being on June 11th. The greatest number of males emerged on June 11th, and of females on June 12th. Of the 3095 adults obtained, 882 were males, 2213 were females, the sex ratio being 28.5 : 71.5. Emergence takes place between about 6.30 a.m.¹ (standard time) and 7.30 p.m., the greatest numbers (70-93 per cent.) appearing between 6.30

¹ All times given are Greenwich standard time.

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and 11 a.m. The males emerge rather earlier than the females, about 33 per cent. of the males hatching before 7 a.m. as against 0.5 per cent. of the females. From 7 a.m. to 9 a.m. is the most favourable time for both sexes. Occasionally there is a second minor crest of emergence between 2 p.m. and 7.30 p.m., which is composed chiefly of males. The eggs are laid between the palae in groups of from one to five. They are a clear shiny yellow and are provided with a pedicel about two-thirds the length of the egg. Without the pedicel the eggs average 0.2608×0.0514 mm. The egg stage appears to last about six days and first stage larvae were obtained on June 15th from eggs laid on June 10th.

Further larvae were obtained from these plants on June 20th and then appeared to be in the second instar. Their average measurements were 0.6832×0.2237 mm. On June 25th larvae were found on a plant which had been infested on June 2nd. Their average measurement was 1.5238×0.4476 mm., and when placed on coconut fibre they went down for pupation.

On July 1st all the heads of infested grasses were cut and brought indoors. They were sprayed with water on July 4th when numerous larvae dropped out. The size of those leaving the plants which had been infested on June 3rd averaged 1.3800×0.4204 mm., while the larvae from plants infested on June 8th and 9th averaged 1.5238×0.3714 mm. All were ready for descending to the soil.

The spraying was repeated on July 9th, when a few more larvae dropped.

The larval period in the inflorescence is therefore from twenty-three to thirty days and may apparently be extended as in the case of *Dasyneura leguminicola*, *D. dactylidis* and *Contarinia dactylidis*.

(c) *Damage and infestation.*

The larvae feed collectively on the ovary which may be quite eaten away by their attack. From samples of heads brought in from the Park Grass Plots in 1931 the following midges and parasites were bred out in 1932:

Samples of 100 heads each	♂♂	♀♀	Parasites	Total	Average per head
RA	151	351	5	507	5.02
RB	41	160	1	202	2.02
RC	154	298	3	455	4.55
RD	132	495	9	636	6.36
RE	235	536	0	771	7.71
RF	143	266	0	409	4.09
RG	14	46	0	60	0.60
				Average	4.33

The infestation is seen to range from 0.60 to 7.71 per head, with an average of 4.33. This again, as the larvae feed collectively, cannot be considered heavy damage since there may be from 120 to 200 flowers in a spike.

(d) *Parasitism.*

Parasitism was extremely low in the 1932 brood, being only 0.75 per cent. (23 parasites—3095 midges), and it is probable that a heavier attack by the midge will be evident in subsequent years. The crest of parasites was on June 3rd, eight days before that of the midges. The parasites involved have been identified by Dr Ferrière as *Inostemma boscii* Jur. [Scelionidae] and a species of Chalcididae.

(e) *Immunity.*

Immunity trials were set up with *Dactylis glomerata*, *F. rubra* var. *arenaria*, and *Alopecurus pratensis*.

The plants were infested with successive batches of midges on June 6th, 7th and 10th. Altogether *D. glomerata* received forty-three females, *F. rubra* thirty-three females and *Alopecurus pratensis* thirty-five females. The heads were all cut on July 1st and brought indoors. They were then sprayed with water at intervals of about four days. No larvae left the heads, and the three species are therefore considered not subject to attack by *C. lolii*.

V. CONTROL.

Hitherto, no record has been made by seed growers in England that the three grass midges described above are responsible for any serious damage. At Harpenden, the combined attack of *Dasyneura dactylidis* and *Contarinia dactylidis* upon Cocksfoot in 1931 was only responsible for the loss of about 0.6 per cent. of the seed. Parasitism in *D. dactylidis* averaged 42.3 per cent., in *C. dactylidis* 11.6 per cent. and in *C. lolii* only 0.75 per cent. in 1932. There are no data to show whether parasitism is on the increase or decrease but it is very low in the case of the two *Contarinia*. It is unfortunate that these observations, begun in 1931, cannot be continued over the next two years, so that it could be estimated whether the pests were increasing or not.

A successful method of combating the attacks of Cecidomyids is to control the period of susceptibility in the host plant so that it has already been passed, or has not yet been reached when the flight of midges is at its maximum. Early cutting of the first crop of clover is advocated so that the second crop may be free from attack (Creel and Rockwood, 1918). Barnes (1930) suggests that grazing of grass fields by sheep so as to

prevent the flowering of the grass until after the crest of emergence of midges as a means of control for *D. alopecuri*, *C. merceri* and *Stenodiplosis geniculati* on *Alopecurus pratensis*. Tomaszewski (1931) states that the grass should be cut from two to three days after the crest of emergence of the midges, and should then be dried and carted with all possible speed. This has the effect of killing the larvae in the very young stages and so diminishes the numbers of the overwintering brood. Both these methods might be applied with success to the control of the Cecidomyids on *Dactylis glomerata* and *L. perenne*.

VI. SUMMARY.

1. Three species of Cecidomyiidae have been observed destroying grass seed on the Park Grass Plots at Harpenden: two species attacking *Dactylis glomerata*, and the third *L. perenne*. Of the two species on *D. glomerata* one has been identified as *Contarinia dactylidis* (H. Lw.), the other is described for the first time as *Dasyneura dactylidis* sp.n. The species present on *L. perenne* is also a new species and is described as *C. lolii* sp.n.

2. All three species of midges are single brooded at Harpenden, the larvae overwintering in the soil. *D. dactylidis* emerges between May 21st and June 6th, *C. dactylidis* between June 3rd and 24th and *C. lolii* between May 31st and June 26th. The damage is done by the larvae which feed on the ovary, singly in the case of *D. dactylidis*, collectively in the other two cases. Parasitism by certain Hymenoptera in 1932 was respectively 42.3, 11.6 and 0.75 per cent.

3. It has not been found possible to cause attack by *C. dactylidis* on *F. rubra* or *L. perenne*. Similarly no infestation by *C. lolii* was produced on *F. rubra*, *Dactylis glomerata* or *Alopecurus pratensis*. It appears probable that these gall midges are specific in their attack.

4. In a brief discussion of control methods it is suggested that delaying the flowering of the grasses either by grazing sheep or by clipping, or by very early cutting to prevent the development of young larvae, might prove effective.

VII. ACKNOWLEDGMENTS.

The work was carried out at the suggestion of Dr H. F. Barnes of the Rothamsted Experimental Station who very kindly placed his collection of literature and gall midges, including types, at my disposal. Prof. Stapledon of the Welsh Plant Breeding Station and Messrs Sutton and Sons of Reading have kindly supplied the grass plants for experiments.

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Owing to the recent publication of Tomaszewski's and Barnes' compilations which contain complete references to the literature on the subject, it is considered sufficient to refer to these papers with a few additional references not given by them:

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STUDIES UPON THE COPPER FUNGICIDES

II. SOME MODIFICATIONS OF BORDEAUX MIXTURE
DESIGNED TO OVERCOME PRACTICAL DIFFICULTIES
IN ITS APPLICATION.

By HUBERT MARTIN.

*(Chemical Research Laboratory, South-Eastern Agricultural
College, Wye, Kent.)*

BORDEAUX mixture, although the most widely used of the copper fungicides, has disadvantages which have caused a demand for a substitute of equal efficiency as a protective fungicide. These disadvantages may be classified under three heads: inconveniences of preparation, difficulties of application and defects connected with its action on the fungus and on the host-plant. The discovery of paste or powder substitutes from which a spray is more easily prepared than is Bordeaux mixture is dependent on, and must probably await, better knowledge of the chemical and physical factors which have made Bordeaux mixture as successful as it is. Similarly, attempts to remove the undesirable features of the action of Bordeaux mixture on fungus and host-plant must be more or less empirical on the basis of our present knowledge of the mechanisms of the fungicidal and phytocidal actions of this spray. The present paper is, therefore, concerned only with the difficulties encountered by the grower in the application of Bordeaux mixture and with certain modifications in the composition of the spray designed to reduce these difficulties. Although the modifications suggested do not result in obviously profound changes in the chemical properties of the Bordeaux precipitate, a preliminary examination has been made of their fungicidal and phytocidal properties by means of field trials, a summary of which is given.

THE APPLICATION OF BORDEAUX MIXTURE.

The present method of applying Bordeaux mixture requires the use of nozzles fitted with a disc (No. 0) with the smallest aperture practicable and adjusted to give a fine mist-like spray. The long run of general experience has shown that, by such a spray, applied in amounts just

insufficient to produce a dripping from the foliage, the maximum fungicidal efficiency of Bordeaux mixture is obtained, coupled with the minimum phytocidal action. It is mainly in the production of this mist-like spray that difficulties are encountered by the grower. Firstly, the wear and tear of the spray pumps is reported to be excessive; secondly, the application of the spray is tiring and time-consuming; thirdly, the spray lacks carrying power; fourthly, it is impossible to incorporate direct fungicides or contact insecticides in the spray. The latter defect is more noticeable on those occasions in routine spraying practice when obvious saving in labour, and especially in time, could be obtained by the use of combined protective fungicide-contact insecticides or of sprays which enable the simultaneous control of two or more fungus parasites each requiring a different treatment. Examples of such occasions occurring in the spraying of fruit are the approximate coincidence of the times of application of sprays for the control of apple scab (*Venturia inaequalis* Aderh.) and the apple capsid bug (*Plesiocoris rugicollis* Fall.) or, again, the correct time of application of contact insecticides against the apple fruit sawfly (*Hoplocampa testudinea* Klug.) approximates to that of the first post-blossom scab spray. In the spraying of hops it would often be possible, if suitable combination sprays were available, to control the hop powdery mildew (*Sphaerotheca humuli* (DC.) Burr.) and the hop-damson aphid (*Phorodon humuli* Schrank) by means of a spray intended primarily for the control of another fungus, the hop downy mildew (*Pseudoperonospora humuli* (Miy. et Takah.) Wils.).

With the present method of applying Bordeaux mixture the addition of contact insecticides or direct fungicides is of doubtful value because the most effective control of Erysiphaceae or of haustellate insects is obtained by the use of a coarse spray applied in amounts sufficient to drench the foliage. For this purpose, discs (Nos. 1 or 2)¹ of relatively large aperture are used in the nozzles. It is convenient to refer to the type of spraying suitable for contact insecticides as "washing" and to the application of Bordeaux mixture and other protective fungicides as "spraying," so to render possible an efficient combined spray, modifications of Bordeaux mixture are required which will permit application of the protective fungicide as a wash.

Modified Bordeaux mixtures which may be applied as washes may also overcome the other difficulties encountered in the spraying of Bordeaux

¹ I am indebted to Mr C. Davies, of the Engineering Department of this College, for the following figures for the average diameter of the apertures of discs, used in commercial practice, which he has examined: that of No. 0 discs is 0.5 mm., that of No. 2 discs is 2.3 mm.

mixture. Wear on the spray pumps is caused mainly by the presence of grit in the lime used; the recent introduction of good grade hydrated limes (7) for stone lime may go far in remedying this defect. Wear on the return valves of the sprays may be reduced, also, if larger quantities of spray pass to the spray nozzles. The time taken to spray a given area will be lessened when a larger volume of spray passes through the nozzles. Another limiting factor in time taken to spray a given area is fatigue on the part of the man performing the spraying. Connected with fatigue there is this psychological factor; in spraying the operator has to beware of overspraying and of underspraying, in washing the need for avoiding overspraying disappears. The elimination of this need for care makes "washing" a less skilled and less tiring task than "spraying." The lack of carrying power of the "spray," which may easily be remedied, in the case of tree spraying, by means of longer and lighter lances, is of more concern to the hop grower. Confronted with the necessity of applying Bordeaux mixture for the control of the hop downy mildew, the only implement available to him was the hop washer, a machine designed for the washing type of spray application. When fitted with No. 0 discs, many machines were unable to spray effectively the higher parts of the hop bine. This difficulty has been solved (4) by the use of an auxiliary motor driving a fan, the spray being carried higher by the draught of air thus produced. It is possible also that by the juxtaposition of the spray nozzles into batteries, the carrying power of the spray would be increased. With the introduction of a modified Bordeaux mixture which can be used as a wash, the need for mechanical alteration of the hop washer would disappear.

The reasons for the inferior fungicidal properties and increased phytocidal action of ordinary Bordeaux mixture when applied as a wash appear to be connected with the character of the deposit left by the spray. When "sprayed" the Bordeaux deposit is in the form of small patches of an area approximating to that of the droplets and in amount equal to the initial concentration of precipitate in the spray. When "washed" large drops are formed in which the precipitate sinks to the lower part and either falls with the drop or remains to give a deposit irregular in distribution and in content of copper. It is probable, also, that the thick deposit left by large drops has inferior adherent properties and flakes off more readily than the deposit left after spraying.

This explanation of the inefficiency of the deposit left after Bordeaux mixture has been "washed" on leads to the suggestion that the addition of a capillary-active material, known in spraying practice as a "spreader,"

might result in a more evenly distributed deposit¹. In the first place, the reduction of surface tension so effected will tend to decrease the volume of the drops formed and the lowering of the contact angle will lead to an increase in the area of leaf covered by a drop of unit volume. The relationship between the area covered by drops of equal volume of liquids of different wetting ability and the contact angle, as measured by the tangent to the drop surface at the point of contact with the surface on which the drop rests, has been investigated mathematically by O'Kane, Westgate, Glover and Lowry(14), who have demonstrated that the lower the contact angle the greater the area of surface covered. Secondly, by the action of the spreader as a protective colloid, the separation of the precipitate to the lower part of the drop will proceed less rapidly and changes in the local concentration of precipitate as the drop dries will be reduced. Thirdly, if a contact insecticide or direct fungicide is to be added to the modified Bordeaux mixture, the presence of a spreader will add to the efficiency of the spray by enabling a better wetting of the insect or by facilitating the penetration of the spray between the conidiophores of a fungus.

On these lines the problem is reduced to the selection of a spreader suitable for addition to Bordeaux mixture. Soap, the spreader widely used for the application of contact insecticides, is inadmissible because interaction with the excess of lime of the Bordeaux mixture results in the precipitation of the soap and the destruction of wetting powers. The addition of gelatine and of casein to Bordeaux mixture has been recommended by Vermorel and Dantony(18), and has been the subject of investigation in the United States (see(9)). Boyd(3) concluded that the addition of lime casein improved the spreading properties, but had no effect on the adhesiveness of Bordeaux mixture applied to potato foliage. Casein and gelatine would, perhaps, interfere with the fungicidal properties of Bordeaux mixture, for it has been suggested that the toxic action of copper is due to a combination with, or adsorption by, the protein of the organism. On this hypothesis, the presence of foreign protein might lead to a decrease in fungicidal activity. There remains available only a limited number of spreaders which are unaffected when

¹ In a recent communication (a), Hockenyo and Irwin have demonstrated the decrease in uniformity and amount of deposit left after the application of quantities of Bordeaux mixture sufficient to cause "running-off." They have further shown that the addition of spreading agents to Bordeaux mixture increases, to some extent, the uniformity of the deposit and eliminates the deposition of heavy spots without decreasing the amount of copper deposited per unit area when the spray is applied in amounts sufficient to give "run-off."

added to Bordeaux mixture and from these, sulphite lye⁽¹¹⁾, a waste product obtained by the concentration of the lye from the sulphite process of wood-pulp manufacture, was selected for investigation, largely on account of its cheapness and its properties as a protective colloid. The first modification of Bordeaux mixture proposed is, therefore, the addition of concentrated sulphite lye to the lime suspension prior to the addition of the solution of copper sulphate.

The second proposed modification of Bordeaux mixture to provide a wash also involves the use of a spreader, but the development has proceeded on different lines. In a survey of the action, on the hop powdery mildew, of solvents suitable as "carriers" for contact insecticides such as nicotine, the pyrethrins or rotenone, Martin and Salmon⁽¹²⁾ showed that glyceride oils have fungicidal properties sufficient to kill the mildew at concentrations of the order of 0.5 to 1 per cent. Convenient methods for the field preparation of sprays containing these oils were required, and one of the most promising depended on the emulsification of the oil by means of Bordeaux mixture, the emulsifying properties of which were discovered by Pickering⁽¹⁵⁾. Not only were washes so prepared effective in the control of the hop powdery mildew⁽¹³⁾, but it seemed worth while to examine the possibility of such a wash being an effective copper fungicide. Further, the toxicity of the oil to the powdery mildew in the absence of a recognised spreader, indicated that the oil was itself able to make good contact with the densely packed conidiophores of the fungus and to act in the same way as a spreader solution. The Bordeaux emulsion of glyceride oil may, therefore, also provide a means for the application of a contact insecticide. Finally, it is possible that the insecticidal properties of vegetable oils emulsified by soap, utilised by Staniland⁽¹⁷⁾, may be shared to some extent by the vegetable oil-Bordeaux emulsion. The second modification of Bordeaux mixture proposed is, therefore, to produce an emulsion by agitation after the addition of a mixture of glyceride oil and stock copper sulphate solution to the lime suspension.

EXAMINATION OF THE MODIFIED BORDEAUX MIXTURES BY FIELD TRIALS.

Before the proposed modifications of Bordeaux mixture can be generally recommended for use in routine spraying, the following points have to be proved. It has to be shown, firstly, that the protective fungicidal properties of the Bordeaux mixture are not reduced by the presence of sulphite lye or glyceride oil and that the deposit left after washing on the modified mixtures is as effective as that left after spraying Bordeaux mixture; secondly, that the application of large quantities of the modified

Bordeaux mixtures does not aggravate the phytocidal properties of Bordeaux mixture; thirdly, that the modified Bordeaux mixtures act effectively as carriers for contact insecticides; lastly, that the application of the modified Bordeaux mixture is easier than that of Bordeaux mixture. The ultimate establishment of these points can only be made on the basis of repeated field trials and will clearly take a long while. A number of field trials have now been carried out by, or in co-operation with, the Mycological and Entomological Research Departments of this College, and a tentative estimate may be made of the extent to which the modified Bordeaux mixtures fulfil these requirements.

In 1931 field work was limited to tests necessary to determine the degree of safety of the washes to the foliage of various crop-plants and trees, and to an examination of the relative efficiencies of the modified Bordeaux mixtures for the control of potato blight. The results were satisfactory and, in 1932, an investigation was begun of the fungicidal action not only against potato blight but also against apple and pear scab and the downy mildew of hop, and of the value of the washes as substitutes for soap solutions as carriers for nicotine for the control of apple fruit sawfly and of the hop-damson aphid.

Supplementary to the field trials, an attempt has been made to investigate special aspects of the fungicidal, insecticidal and phytocidal properties of the washes in the laboratory in order to obtain pointers of value in the interpretation of the results of the field trials. For example, the protective fungicidal action of copper sprays is due, unless an indirect action is assumed by which the host-plant is rendered immune from disease, to the slow formation of an active fungicide from the deposit left by the spray. The efficiency of the protectant will, therefore, be dependent on factors which govern the retention of the deposit as well as on factors which concern the formation of the active fungicide. A knowledge of the relative amounts of copper in the deposit retained after spraying and the differences in the retention of this deposit is therefore necessary for the examination of differences in the fungicidal properties of the various modifications of Bordeaux mixture. Indications of differences in the adherence of the deposit can be obtained if a suitable technique is available for the estimation of the amount of copper retained on the sprayed foliage. A description of the method adopted for this purpose follows the discussion of the results of the field trials.

Potato blight trial, 1931.

This trial was carried out in co-operation with Mr M. D. Austin and a full report has been published (2). The plot of King Edward potatoes was divided into sixteen sub-plots forming a Latin square of four treatments: (1) unsprayed, (2) 10 : 15 : 100 Bordeaux mixture, employing 15 lb. hydrated lime per 100 gallons, (3) 10 : 15 : 100 Bordeaux mixture plus 0.75 per cent. concentrated sulphite lye (60° Tw.) and 0.02 per cent. nicotine, (4) 1 per cent. cottonseed oil containing the light petroleum extract equivalent to 1 per cent. ground pyrethrum flower heads, emulsified with 10 : 15 : 100 Bordeaux mixture. The dates of application of these sprays and the estimates of the amount of copper retained, expressed as milligrams of metallic copper per square metre of leaf surface (neglecting the under side of the leaves), are given in Table I. The results of duplicate estimations are bracketed together. Sampling of the leaves for these estimations presented no difficulty, except that in the sampling made on July 29th, 1931, only leaflets from the lower leaves of the plants were taken, one leaflet being selected at random from plants about 6 ft. apart. Samples taken from plots of the same treatment were mixed before cutting the discs.

Table I.

Spray	1st application 13. vii. 31		2nd application 31. vii. 31		3rd application 18. viii. 31
	16. vii.	29. vii.	31. vii.	17. viii.	i. x.
Bordeaux mixture (10 : 15 : 100)	73 }	41 }	116 }	30 }	48 }
	119 }	24 }	135 }	45 }	48 }
Bordeaux-sulphite lye	84 }	15 }	149 }	37 }	48 }
	95 }	50 }	180 }	49 }	48 }
Oil-Bordeaux (1 : 10 : 15 : 100)*	136 }	159 }	100 }	105 }	103 }
	208 }	137 }	149 }	105 }	112 }

* The designation "oil-Bordeaux" is used to describe the oil spray containing Bordeaux mixture as the emulsifier in order to distinguish this wash from the combination of Bordeaux mixture and oil emulsion prepared with casein or other emulsifier used in America and commonly called "Bordeaux-oil." In describing the concentrations used for the preparation of oil-Bordeaux, 1 : 10 : 15 : 100 indicates that the wash contains 1 gallon oil, 10 lb. bluestone, 15 lb. hydrated lime in 100 gallons.

The difference in the amount of deposit retained on leaves washed with oil-Bordeaux and on leaves sprayed with Bordeaux mixture was clearly observable to the eye. There was, however, no observable differences in the action of the three sprays in preventing defoliation of the haulm by blight, nor did the weight of tubers lifted show significant differences due to treatment.

Potato blight trial, 1932.

Because of the improved retention of deposit produced by the incorporation of cottonseed oil in the Bordeaux mixture, a trial was made in which the concentration of the Bordeaux mixture was reduced by half to 5 lb. bluestone, and 7.5 lb. hydrated lime per 100 gallons. The oil concentration was also reduced to 0.75 per cent. by reason of successful trials with this oil concentration on the hop powdery mildew (13).

A plot of King Edward potatoes was divided into twenty-five equal sub-plots and the following treatments were randomised on the basis of a Latin square: (1) unsprayed, (2) 10:15:100 Bordeaux mixture, (3) 10:15:100 Bordeaux mixture plus 0.75 per cent. concentrated sulphite lye (60° Tw.) and 0.02 per cent. nicotine, (4) cottonseed oil containing the light petroleum extract of ground pyrethrum flower heads, emulsified with 10:15:100 Bordeaux mixture, the wash containing 0.75 per cent. cottonseed oil and 0.002 per cent. pyrethrins, (5) 0.75 per cent. cottonseed oil emulsified with 5:7.5:100 Bordeaux mixture and 0.02 per cent. nicotine.

Table II.

Spray	1st application, 18. vii. 32			2nd application, 11. viii. 32		
	Amount applied (gall.)	mg. Cu/sq. m. upper leaf surface		Amount applied (gall.)	mg. Cu/sq. m. upper leaf surface	
		20. vii.	5. viii.		13. viii.	24. viii.
Bordeaux mixture (10:15:100)	18	230 } 195 }	56 } 45 }	19	130 } 116 }	87 } — }
Bordeaux-sulphite lye (10:15:100)	24	186 } 141 }	44 } — }	28	109 } 124 }	104 } 104 }
Oil-Bordeaux (0.75:10:15:100)	24	203 } 212 }	120 } 117 }	27	170 } 170 }	127 } 135 }
Oil-Bordeaux (0.75:5:7.5:100)	26	97 } 80 }	90 } 76 }	32	90 } 90 }	72 } 80 }

The dates of applications, the quantity applied and the amounts of copper remaining on the leaves are shown in Table II. The enhanced retention of spray deposit on leaves washed with the oil-Bordeaux emulsions was again shown. In the case of the half-strength Bordeaux emulsion the initial amount of copper in the spray deposit was less, but after an interval of 10–20 days the amount remaining was equal to or greater than that on the leaves sprayed with the 1 per cent. Bordeaux mixture.

No differences were observed in the spread of potato blight in the sprayed plots, but the unsprayed haulm died down 3 or 4 weeks before that of the sprayed plots showed signs of defoliation. Significant dif-

ferences were not found in the weight of tubers, either total or ware, lifted from each plot.

Apple scab trials, 1932.

(1) *On Bramley's Seedling.* (With M. D. Austin.)

A plot of forty-two apple trees, six of Newton Wonder and the remainder of Bramley's Seedling, in an isolated position of the College Farm, was placed at our disposal. The trees, which were about 25 years old, had been neglected, and required heavy pruning to bring them into a condition suitable for spray trials. They had been used during the two previous dormant seasons for winter-wash trials and, for this purpose, had been divided into four blocks, three treatments being randomised on the three rows of each block. The winter washes applied on January 26th, 1932, consisted of a mixture of 4 per cent. strained anthracene oil and 4 per cent. semi-refined petroleum oil of a light lubricating type emulsified, in one treatment, by the two-solution oleic acid method (10) and, in the second treatment, with 4 : 6 : 100 Bordeaux mixture. A severe aphid attack developed on the unsprayed trees, to eliminate which a wash, containing 6 oz. nicotine per 100 gallons of a soap solution prepared by the solution of 3 lb. oleic acid in 100 gallons of water containing 1 lb. flake caustic soda, was applied on April 30th.

For the spring washes, the same arrangements of plots was used; the trees winter-washed with oil-Bordeaux emulsion were washed with 1 per cent. crude expressed oil of mustard emulsified with 4 : 6 : 100 Bordeaux mixture; to the trees winter-washed with the oleic acid emulsion, 8 : 12 : 100 Bordeaux mixture containing 0.75 per cent. concentrated sulphite lye was applied; the trees unsprayed during the dormant season were sprayed with 8 : 12 : 100 Bordeaux mixture alone. The crude expressed oil of mustard used had an acid value equivalent to 27.3 gm. oleic acid per 100 ml., and the concentration of 1 per cent. was chosen to give approximately the same glyceride content as in the 0.75 per cent. cottonseed oil-Bordeaux emulsions used in other trials during 1932. The presence of this large amount of free fatty acid did not affect the emulsification of the oil of mustard by Bordeaux mixture, but a scum of copper and calcium soaps separated which, although preventing the use of a strainer, did not cause trouble by blocking the nozzles during the application of the wash.

Three applications were made for the control of apple scab, one pre-blossom and two post-blossom. Arsenate of lead paste was added, at the rate of 4 lb. per 100 gallons to the Bordeaux mixture and to the Bordeaux-

sulphite lye wash and at the rate of 2 lb. per 100 gallons to the oil-Bordeaux wash, in the first post-blossom application, as a precautionary measure against codling moth. Three trees, one in each treatment, were left unsprayed to serve as indicators for the control of scab given by the copper fungicides. The sprays were applied by means of a small power sprayer with two lances, the nozzles of which were fitted with No. 0 discs for the application of Bordeaux mixture and with No. 1 discs for the application of two modified Bordeaux washes, which were applied in amounts sufficient to drench the foliage. Weather conditions were perfect for each application except the first, which was begun on May 9th. Before the Bordeaux mixture, which was the first spray to be applied, had dried on the trees, heavy rain fell and this treatment had to be repeated when the other sprays were applied on May 10th.

Leaf samples were taken as soon as possible before and after each application and, prior to the first post-blossom application, leaves were gathered only from blossom trusses. The method of selecting the leaves was to ignore the first leaf showing spray deposit seen on approaching the branch and to take the leaf adjacent to it on the blossom truss or lam-bourde or the leaf next below on the new wood. It is possible that an error is introduced by neglecting, with trees of this size, to sample leaves from the top branches. By taking a proper proportion of leaves from outside and within the tree, the seriousness of this error was reduced to a minimum. The amount of copper expressed in milligrams per square metre on one side of the leaf, the average amount of spray applied per tree and the dates of application of sprays and of leaf samplings are shown in Table III.

Table III.

Spray	Pre-blossom application, 10. v. 32		1st post-blossom application, 1. vi. 32			2nd post-blossom application 20. vi. 32					
	Gall. per tree	mg. Cu/sq. m. leaf surface		Gall. per tree	mg. Cu/sq. m. leaf surface		Gall. per tree	mg. Cu/sq. m. leaf surface			
		13. v.	27. v.		3. vi.	18. vi.		22. vi.	16. vii.	31. vii.	18. viii.
Bordeaux mixture (8 : 12 : 100)	1.75	124	106	1.25	133	57	1.5	84	99	85	74
		124	85		169	67		124	124	81	74
Bordeaux-sulphite lye (8 : 12 : 100)	2.5	85	65	2.4	96	67	2.5	121	106	81	78
		82	81		108	71		149	115	97	88
Oil-Bordeaux (1 : 4 : 6 : 100)	3.2	87	85	2.3	81	57	2.8	85	67	67	60
		81	83		55	57		85	87	71	53

There are no striking differences in the amounts of copper retained in the spray deposit, and it is apparent that, under the conditions of the trial, the addition of sulphite lye has not given a deposit more easily removed by rain or mechanical agencies than that given by Bordeaux

mixture. The initial concentration of copper on leaves washed with the oil-Bordeaux emulsion is smaller than that on leaves sprayed with Bordeaux mixture or washed with Bordeaux mixture and sulphite lye, but the differences tend to disappear with time.

Scab was not conspicuous on the sprayed trees until 4-6 weeks before fruit picking, when isolated leaf and fruit infections were observed. On the unsprayed trees, scab became general on the foliage, but the fruit remained fairly free until late in the season. At fruit picking, October 3rd-6th, some apples from the unsprayed trees were sufficiently disfigured and distorted by scab to be considered unsaleable, but the number was not enough to warrant a special grade. An apple was placed into the scabbed grade if it bore an area of scab infection greater than the equivalent of a circle of $\frac{1}{8}$ in. diameter. Apples with noticeable skin blemish were counted and those on which russetting was serious enough to be likely to affect their market value were classed as "severe" in Table IV, which gives the number of apples picked and the percentage of scabbed fruit per row, according to treatment.

Table IV.

Spray	Row	Number of apples	%	% unscabbed fruit with skin blemish	
				Slight	Severe
Bordeaux mixture (8:12:100)	12	931	22.2	26.9	2.3
	7	1634	20.6	23.3	1.1
	6	1611	21.7	19.4	0.8
	2	1283	9.4	35.6	2.3
	Total	5459	Av. 18.6	26.1	1.5
Bordeaux mixture (8:12:100) plus sul- phite lye (0.75 %)	10	631	7.6	22.1	3.8
	8	2120	10.8	22.6	3.1
	4	1741	11.3	26.0	2.1
	3	1587	11.7	30.4	1.2
	Total	6079	Av. 10.9	25.5	2.4
Oil-Bordeaux (1:4:6:100)	11	2926	15.6	23.2	4.0
	9	713	16.7	9.0	0.5
	5	696	4.9	16.9	2.0
	1	1674	26.9	28.2	2.5
	Total	6009	Av. 17.6	21.9	3.1
Unsprayed	Tree in row			% total fruit with skin blemish	
				Slight	Severe
Unsprayed	9	312	97.8	10.6	0.0
	6	350	91.7	12.3	0.0
	4	375	95.7	9.1	0.0
	Total	1037	Av. 95.0	10.6	0.0

Good control of scab was given by the three washes used and, if the unsprayed trees be ignored, statistical examination of the figures for percentage of scabbed apples per row reveals no significant difference due to treatment; variance due to treatment = 69.48, that ascribable to error = 56.88.

The actual number of apples with skin blemishes is greater on the sprayed trees than on the unsprayed, but it is impossible to attribute all, especially those with corky roughness at the stalk end of the apple, to the results of spray application. The number of severely russeted apples is relatively small and there is no significant difference due to type of fungicide used.

(2) *On Newton Wonder and Allington Pippin.*

This trial was carried out in co-operation with Dr W. Goodwin, Prof. E. S. Salmon and Mr W. M. Ware, and a full account will be published (5).

A plantation of forty-two trees of Newton Wonder and thirty-six trees of Allington Pippin, planted in 1913, was divided into two main plots with three smaller unsprayed plots distributed in the plantation. For the control of scab, the trees of the first plot were sprayed with 8 : 12 : 100 Bordeaux mixture, those of the second plot being washed with 1 per cent. crude expressed oil of mustard emulsified with 4 : 6 : 100 Bordeaux mixture. Two pre-blossom and two post-blossom applications were made and arsenate of lead paste was added to both the pre-blossom sprays, at the rate of 4 lb. per 100 gallons to the Bordeaux mixture and 2 lb. per 100 gallons to the oil-Bordeaux emulsion. To keep down woolly aphis, which had been numerous in previous years, nicotine (8 oz. per 100 gallons) was added to the second pre-blossom and first post-blossom sprays. The trees unsprayed against scab were washed with an insecticide containing 6 or 8 oz. nicotine, 4 lb. lead arsenate paste and 6 pints of concentrated sulphite lye at approximately the same time as the first and second pre-blossom scab sprays.

A small power sprayer and two lances were used for the application of the sprays, and the nozzles were fitted with No. 0 or No. 1 discs according to the type of spray and time of application. The average time taken per tree, the average amount applied per tree, the date of application and disc used, and the results of copper estimations and area of leaf surface are included in Table V.

Although the number of trees sprayed was too small to permit an accurate comparison of the times taken to spray and to wash the trees, it is perhaps significant that, in every case, washing took less time than

Table V.

[illegible]

spraying. The estimation of copper remaining on the leaves was again complicated by the size of the trees, and the same precautions were taken as in the trial on Bramley's Seedling already mentioned. Prior to the post-blossom sprays, leaf samples were taken from blossom-truss buds only. Except in the case of the pre-blossom application, more copper is present initially on trees sprayed with Bordeaux mixture and the relative differences between the amounts of copper on the Bordeaux and oil-Bordeaux leaves do not decrease with time to the same extent as with potato leaves washed with cottonseed oil-Bordeaux emulsion and sprayed with Bordeaux mixture. It is possible that the copper soap formed from the free fatty acids of the crude oil of mustard may contribute to the initial amount of copper on the leaves washed with oil-Bordeaux and that, lacking adhesive properties, this form of copper is rapidly lost, thereby increasing the ratio of copper initially present to that present after a given time and reducing the amount of copper applied as Bordeaux precipitate.

A comparison of copper retention on the basis of unit area of leaf surface involves the assumption that the relative growth of leaves treated with the different fungicides remains constant. Thus it might be suggested that the greater retention of spray deposit per unit area on leaves washed with oil-Bordeaux emulsion compared to that of the Bordeaux deposit is due to a greater growth in surface area of the leaves sprayed with Bordeaux mixture. This suggestion was examined by the estimation of the surface area of samples of fifty leaves gathered from each plot. This figure was determined from the total weight of leaves without stalks and the weight of discs of known area cut from the leaves. Sampling of the leaves for this purpose was made difficult by differences due to biennial bearing, but the estimates, given in Table V, in square metres covered by fifty leaves, show that no significant increase in area had occurred in 5 weeks and that the average surface area of the leaves of trees washed with oil-Bordeaux was significantly greater than that of those sprayed with Bordeaux mixture. This difference is more likely to be due to position than to treatment.

At fruit picking, the differences between the percentages of clean fruit, that is, with scab infections of an area not greater than that of three circles of 2 mm. diameter, obtained from the sprayed and unsprayed plots, were as follows: of the Newton Wonder apples, 85.9 per cent. by number from the Bordeaux plot and 49.8 per cent. from the oil-Bordeaux plot; of the Allington Pippins, 73.1 from the Bordeaux plot and 62.5 per cent. from the oil-Bordeaux plot. Counts were also made of the number of

apples sufficiently russeted to be considered of lower market value; of the Newton Wonder apples 2.8 per cent. russeted were obtained from the Bordeaux plot and 0.6 per cent. from the oil-Bordeaux plot; of the Allington Pippins, 2.9 per cent. of the apples picked from the Bordeaux plot were russeted and 1.6 per cent. from the oil-Bordeaux plot.

It is evident that, on the Newton Wonder trees, the oil-Bordeaux wash has not controlled scab as well as the Bordeaux mixture, but, on the Allington Pippins the difference is not so large. If it be assumed that similar differences would have been obtained had the trees washed with oil-Bordeaux emulsion been sprayed with Bordeaux mixture and *vice versa*, the differences are to be attributed, in view of the results of the trial on Bramley's Seedling, to the smaller amount of copper retained on foliage washed with oil-Bordeaux and not to a reduction of fungicidal efficiency of the Bordeaux deposit caused by the presence of oil. It may also be concluded that the smaller percentages of russeted apples of both varieties picked from the oil-Bordeaux plot are associated with the smaller amount of copper retained on the foliage.

Apple fruit sawfly trial, 1932.

This trial formed part of an investigation of various insecticidal washes for the control of apple sawfly, carried out in co-operation with Messrs M. D. Austin and S. G. Jary, a full account of which has already been published (1).

Blocks of six trees of the varieties Early Victoria, Grenadier and Newton Wonder were treated with the following washes: (1) nicotine soap, containing 8 oz. nicotine per 100 gallons of a soap solution prepared by the solution of 3 lb. brown commercial oleic acid in 100 gallons of 0.1 per cent. sodium hydroxide solution; (2) Bordeaux-sulphite lye-nicotine, in which 8 oz. nicotine were added to 100 gallons of 8 : 12 : 100 Bordeaux mixture containing six pints of concentrated sulphite lye (60° Tw.); (3) oil-Bordeaux emulsion nicotine, to prepare which 8 oz. nicotine were added to an emulsion of six pints of a cheap grade of edible cottonseed oil in 4 : 6 : 100 Bordeaux mixture. The washes were applied by means of a small power sprayer and two lances of which the nozzles were fitted with No. 2 discs. The date of application was determined on the basis of observations of the hatching of sawfly eggs on neighbouring trees. The trees, about 10-12 ft. high and well pruned out, were drenched with the washes and the average amounts applied per tree were as follows: nicotine soap, 1.92 gallons, Bordeaux-sulphite lye nicotine, 1.75 gallons and of oil-Bordeaux emulsion nicotine, 2.92 gallons.

Not the slightest damage to the trees was observed as the result of the heavy applications of the modified Bordeaux mixtures.

Sawfly attack, which was estimated by counts of total and marked fruitlets on branches selected at random, did not develop to any noteworthy extent on the varieties Early Victoria and Grenadier. From counts on the Newton Wonder trees the following percentages of marked fruitlets were obtained: on the unsprayed trees, 27.9 per cent. of a total of 308; on the trees washed with nicotine soap, 14.9 per cent. of a total of 87; on the trees washed with the Bordeaux-sulphite lye nicotine combination, 11.1 per cent. of a total of 144; on the trees washed with the oil-Bordeaux emulsion, 15.0 per cent. of a total of 272. The degree of sawfly control obtained with combinations of nicotine and the modified Bordeaux mixtures is comparable with that obtained by the use of a soap nicotine wash.

Pear scab trial, 1932.

For this trial, carried out in co-operation with Dr W. Goodwin, Prof. E. S. Salmon and Mr W. M. Ware (6), a block of pear trees was placed at our disposal by Mr T. Neame, of Faversham, to whom we are also indebted for the provision of spraying tackle and labour.

The block, which contained trees of the varieties Beurré d'Amanlis, Dr Jules Guyot, Louise Bonne and Beurré Hardy, was divided into two plots by a guard row running at right angles to the direction of the variety rows. One plot, of 119 trees, was sprayed with Bordeaux mixture (8:12:100) and the other, of 120 trees, was washed with cottonseed oil-Bordeaux emulsion (0.75:4:6:100). Five applications of the sprays were made, two pre-blossom and three post-blossom and, to the pre-blossom sprays, arsenate of lead paste was added for the control of winter moth. Particulars of the dates of applications, nozzles and discs used, average amount applied per tree and the time taken per man are shown in Table VI.

Table VI.

	Pre-blossom applications				Post-blossom applications					
	First		Second		First		Second		Third	
	Bor-deaux	Oil-Bordeaux	Bor-deaux	Oil-Bordeaux	Bor-deaux	Oil-Bordeaux	Bor-deaux	Oil-Bordeaux	Bor-deaux	Oil-Bordeaux
Date applied	7. iv. 32		19. iv. 32		17. v. 32		2. vi. 32		28. vi. 32	
Nozzle used	Double		Double		Single	Double	Single	Single	Single	Single
Disc used	Two No. 1		One No. 0 and one No. 1		No. 0	Two No. 1	No. 0	No. 1	No. 0	No. 1
Gall. per tree	1.7	2.0	1.5	1.7	1.4	2.4	1.1	1.9	1.1	1.8
Time per tree per man (min.)	1.4	1.5	1.6	1.8	2.0	1.2	1.5	1.2	2.3	1.4

When the times taken for one man to spray one tree with Bordeaux mixture are compared with those taken to apply the oil-Bordeaux, there is a marked reduction in all the post-blossom applications made. For the pre-blossom sprays the same nozzles were used on each plot, and the slightly longer time taken to apply the oil-Bordeaux is in proportion to the greater amount of spray used.

Estimates of the amount of copper retained on the leaves are given in Table VII. In general, the copper content of the deposit on foliage washed with oil-Bordeaux is of the same order as that on leaves sprayed with Bordeaux mixture, although the copper content of the former spray was but half that of the Bordeaux mixture. Estimates of the surface area covered by fifty leaves are included, for they show that there is no significant difference in the size of leaves of the same variety but of different treatment.

Table VII.

Spray	1st post-blossom applied 17. v. 32 mg. Cu/sq. m. leaf surface		2nd post-blossom applied 2. vi. 32		3rd post- blossom applied 28. vi. 32
			mg. Cu/sq. m. leaf surface		mg. Cu/sq. m. leaf surface
	17. v.	1. vi.	21. vi.	Surface area of 50 leaves (sq. m.) 21. vi.	3. viii.
Beurré d'Amanlis:					
Bordeaux mixture	212 }	144 }	124 }	0.184 }	170 }
	163 }	141 }	97 }	0.187 }	113 }
Oil-Bordeaux	159 }	144 }	103 }	0.179 }	155 }
	149 }	144 }	141 }	0.172 }	177 }
Dr Jules Guyot:					
Bordeaux mixture	163 }	72 }	110 }	0.132 }	248 }
	170 }	72 }	139 }	0.126 }	223 }
Oil-Bordeaux	170 }	96 }	88 }	0.138 }	177 }
	138 }	84 }	97 }	0.128 }	209 }
Louise Bonne:					
Bordeaux mixture	212 }	120 }	122 }	0.095 }	230 }
	191 }	— }	142 }	0.093 }	265 }
Oil-Bordeaux	187 }	133 }	177 }	0.082 }	198 }
	163 }	— }	141 }	0.083 }	230 }
Beurré Hardy:					
Bordeaux mixture	120 }	120 }	159 }	0.193 }	248 }
	198 }	96 }	124 }	0.183 }	269 }
Oil-Bordeaux	184 }	114 }	97 }	0.180 }	230 }
	134 }	144 }	85 }	0.165 }	223 }

The crop was not graded for scab, but inspection of the trees showed that, on both plots, there was very little scab infection and of quite insignificant importance except, perhaps, on the variety Beurré Hardy. On this variety scab occurred on the fruit to a small extent equally in the Bordeaux and oil-Bordeaux plots. No damage was caused by either

spray or wash, excepting a slight leaf fall in the variety Beurré Hardy, to the same degree in both plots.

Hop aphid trials, 1932.

To obtain indications of the relative toxicity of nicotine applied with the two Bordeaux modifications and with soap, small scale trials were carried out on selected hop leaves carrying a suitable aphid population. Full particulars of the technique adopted have already been published (1). A summary of the results obtained are given in Table VIII, in which sprays applied at the same time and under the same conditions are bracketed together. A direct comparison may be made between such bracketed experiments and an indirect comparison between different series is facilitated by the use of a standard nicotine soap preparation (0.00625 per cent. nicotine and 0.13 per cent. sodium oleate in distilled water).

Table VIII.

Spray	Number of aphides treated	% killed
{ Nicotine-soap	2554	98
{ Cottonseed oil-Bordeaux emulsion (0.75 : 4 : 6 : 100) and 0.00625 % nicotine	819	98
{ Cottonseed oil-Bordeaux (0.75 : 4 : 6 : 100)	1300	92
{ Nicotine-soap	606	98
{ Cottonseed oil-Bordeaux emulsion (0.75 : 4 : 6 : 100) and 0.00625 % nicotine	1554	100
{ Cottonseed oil-Bordeaux (0.75 : 4 : 6 : 100)	1562	78
{ Nicotine-soap	723	98
{ Bordeaux (8 : 12 : 100), 0.75 % sulphite lye and 0.00625 % nicotine	1292	97

The results indicate that the two modifications of Bordeaux mixture merit further trial as "carriers" for nicotine. As in unsprayed controls the percentage of aphides killed was negligible, it would appear that the cottonseed oil-Bordeaux emulsion has a definite insecticidal action on this aphid.

Further field trials were carried out with the cottonseed oil-Bordeaux emulsion. In one case, forty hills of the variety Canterbury Golding were treated with 0.75 per cent. of a cheap grade of edible cottonseed oil emulsified with 4 : 6 : 100 Bordeaux mixture containing 0.0375 per cent. nicotine. The more vigorous of the bines had just reached the top wire when washed in hot sunshine on June 17th. The wash was applied in a quantity approximating to 120 gallons per acre, but as the application was made with a knapsack sprayer instead of a hop washer, a concentra-

tion of the wash on the hop plants was possible and the actual dosage given was greater than this figure. No scorching injury resulted from the heavy application although slight "pin-hole" injury developed. The grower reported, however, that this injury was less severe than that caused, on neighbouring hills, by a light application at about the same time of 8 : 12 : 100 Bordeaux mixture.

Hop downy mildew trial, 1932.

Six rows of miscellaneous varieties of hop, of about 180 yards length, were washed with cottonseed oil-Bordeaux emulsion (0.75 : 5 : 7.5 : 100) by means of a tractor-drawn hop washer fitted with No. 1 discs. The first application was made in bright sunshine but rather cool conditions on July 19th, and a second application was made on August 5th in hot, thundery weather. On both occasions the quantity of wash applied was about 450 gallons per acre. No Bordeaux-blower (4) was used, but the spray was carried well over the top wire although slight skidding of the washer wheels, on the second occasion, made irregular the pressure generated by the pumps. No damage was caused to the hop foliage or tips and, possibly because of the hot August, no downy mildew was found on the washed plot.

CONCLUSIONS BASED ON THE FIELD TRIAL RESULTS.

It would be fallacious to deduce general conclusions on the results of field trials continued for only 1 or 2 years, but it is possible to say that, under the conditions prevailing in 1931 and 1932, the modifications of Bordeaux mixture examined have fulfilled, to the following extent, the requirements outlined in the introductory paragraph to the account of the field trials described above. With regard to the fungicidal efficiency of the modified Bordeaux mixtures, the sulphite lye modification has given a control of potato blight and apple scab equal to that given by Bordeaux mixture of a similar copper and lime content. With the glyceride oil-Bordeaux emulsions, because of the increased persistence of the deposit retained on the leaves, the amounts of copper sulphate and hydrated lime used to emulsify the oil were reduced to half those used for the preparation of the Bordeaux mixture. At these concentrations the cottonseed oil-Bordeaux emulsion was as effective as Bordeaux mixture in controlling potato blight and pear scab. The crude expressed oil of mustard-Bordeaux emulsion gave, in one trial, as complete a control of apple scab as that given by Bordeaux mixture but was less effective in another trial. The reduction of fungicidal efficiency was, possibly, due to the formation of a

copper soap in a form easily removed by external agencies rather than to the presence of oil.

The modifications of Bordeaux mixture were applied after the manner of and in amounts comparable to those used in the application of contact insecticides without causing injury to the foliage greater than that produced by lighter applications of Bordeaux mixture. They have proved equal in soap solutions in promoting the insecticidal action of nicotine on the hop aphid and on the apple fruit sawfly. Finally, by enabling the use of a larger volume of spray, they may be applied more easily and more rapidly than Bordeaux mixture.

ESTIMATION OF COPPER RETAINED ON FOLIAGE.

The method adopted for the determination of the amount of copper retained on foliage sprayed or washed with the various fungicides used was as follows. Leaf samples were taken in the field and precautions, dependent on the particular conditions of the trial and described above, were taken to ensure a random selection of sprayed leaves. Circles of known diameter were cut from the leaves, while still fresh, by means of a cork-borer. As the spray deposit is usually more evident on the upper surface of the leaf, the leaf was held with the lower surface uppermost and the disc cut at random, avoiding the edge of the leaf. It was found that by taking samples of two lots, each of fifty leaves, results were obtained of sufficient concordance to show differences likely to be of practical importance. The sample of fifty discs, placed in a porcelain crucible, was dried in a steam oven and ashed over a Bunsen burner, care being taken to remove the flame as soon as the leaf tissue was alight in order to avoid high temperatures likely to cause a loss of copper by volatilisation. Ashing was completed by placing the crucible in an electric muffle for 20 min. Constant temperature conditions were maintained by regulating the current heating the oven at 5 amperes, which gave a dull red heat. When cool, the ash was dissolved in 2 ml. concentrated nitric acid and transferred, by washing, to a small beaker. After the addition of excess ammonium hydroxide and coagulation of the precipitate by standing for a few minutes on a hot plate, the solution was filtered, filter paper and beaker being washed with dilute ammonia. The filtrate was adjusted to a definite volume and a suitable aliquot, dependent on the amount of copper likely to be present, was evaporated almost to dryness, acidified with eight drops of 10 per cent. acetic acid and transferred to a Nessler tube. The amount of copper present was determined colorimetrically by the ferrocyanide method⁽¹⁶⁾, the colour being matched by

the addition of freshly prepared standard copper sulphate solution to a blank obtained by the ashing of unsprayed leaves.

To ensure that the destruction of organic matter by ashing under the conditions described did not result in a loss of copper, a number of comparative estimations were made in which the organic matter was destroyed by wet oxidation. After soaking overnight in concentrated nitric acid, the discs were treated with 10 ml. concentrated sulphuric acid and placed on a hot plate until the appearance of white fumes. Oxidation was continued by the addition of sodium nitrate crystals until the fuming sulphuric acid solution remained colourless. Excess of sulphuric acid was expelled by further heating on the hot plate and the crystalline residue was treated in the same way as the ash. The following are typical results which show the type of agreement obtained in replicate trials, using the dry and wet methods for destroying the organic matter of sprayed pear and apple leaves:

Method of oxidation	Copper (mg.) per sq. m. of upper leaf surface						
	Apple leaves					Pear leaves	
Dry	19	36	73	99	124	120	144
Dry	21	39	83	99	132	96	144
Wet	18	30	81	92	125	120	156

SUMMARY.

1. To overcome difficulties encountered in the application of Bordeaux mixture as a fine mist-like spray, two modifications are proposed which may be applied in larger quantities after the manner of an insecticidal wash. By this means the application of the protective fungicide is made easier and more rapid, the wear on the spray pumps may be reduced and difficulties due to the lack of carrying power of the spray are removed. Further, the incorporation of a contact insecticide with the protective fungicide becomes possible, with resultant saving of labour and of time in the routine spraying of fruit and hops.

2. The modifications of Bordeaux mixture proposed are (1) the incorporation of concentrated sulphite lye in the Bordeaux mixture, (2) the glyceride oil emulsion produced by the use of Bordeaux mixture as emulsifier. As examination of the amounts of copper retained on foliage after spraying showed that the presence of oil improves the retention of spray deposit, the content of copper sulphate and lime of the oil-Bordeaux emulsion was reduced to half that of the Bordeaux mixture used in comparisons of fungicidal efficiency by field trials.

3. The results of field trials show that, under the conditions prevailing in 1931 and 1932, (i) the modified Bordeaux mixtures, applied in heavy amounts, proved as effective as lighter applications of Bordeaux mixture for the control of potato blight, apple and pear scab. In one trial, in which vegetable oil of high free-fatty acid content was used, the fungicidal efficiency of the oil-Bordeaux emulsion was below that of Bordeaux mixture containing double the amount of copper sulphate and lime; (ii) the application of large quantities of the modified Bordeaux mixtures did not cause greater leaf injury or fruit russetting than Bordeaux mixture used in smaller amounts; (iii) combinations of nicotine with the modified Bordeaux mixtures gave washes of insecticidal efficiency equal to that of nicotine-soap wash; (iv) as a result of the use of a spray of larger volume, the application of the modified Bordeaux mixtures was easier and more rapid than that of Bordeaux mixture.

4. A method is described for the estimation of copper retained on sprayed foliage.

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REVIEW

Principles of Soil Microbiology. By SELMAN A. WAKSMAN. 2nd edition. Pp. xxviii + 894, 15 plates, 83 figures in text. Baillière, Tindall and Cox, 8 Henrietta Street, Covent Garden, London, W.C.2, 1931. 52s. 6d. net.

The first edition of Prof. Waksman's *Principles* was reviewed in this *Journal*, xv, No. 1, 1928. The second edition incorporates the new work since 1927, and in order to keep the volume of the same size a great amount of condensation has been practised. The original ground plan has been maintained but a number of chapters have been rewritten, especially those dealing with the mycorrhiza fungi and the soil as a medium for plant and animal parasites. A number of new chapters have been added dealing with the rôle of micro-organisms in the decomposition of organic matter in green manures and stable manures, in the decomposition of peat and forest soils, and with the relation between plant growth and the activities of micro-organisms in soil. The bibliography of soil microbiology grows apace and, whereas in the first edition the author index occupied 21 pages, it fills over 27 pages in the new edition. In order to avoid sacrificing references the author has in the new edition omitted the titles of papers, and this is a serious loss. One feels that it might have been better to make a more stringent selection or preferably to increase the size of the volume by a few pages. Actually the second edition has three pages less than the first edition and 15 instead of 19 plates. As none of the plates is necessary or really adds to the value of the book this space and costs of production might have been devoted to reference titles. Every serious student will find numerous questions on which to disagree in Prof. Waksman's treatise, questions often of major importance but, looking at the work from the larger point of view, one simply accepts it thankfully, gratefully acknowledging the encyclopaedic mind and almost superhuman industry which have gone to its making. It is the only treatise of its class available, a book which every working microbiologist must use and curse daily, and of which a personal copy is quite imperative in spite of its high price. The printing on Figs. 21 and 50 might with advantage have been enlarged, and misprints have been noted on pages 50, 135, 209, 230, 238, 239, 240, 242, 249, 259, 314, 560, 671, 707, 784, 791, 862, 864, 872, 874, 877, 886 and 888.

WILLIAM B. BRIERLEY.

REPORT OF THE COUNCIL OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS FOR THE YEAR 1932

DURING 1932 the Association has met on five occasions, including one field meeting.

This was held at the Biological Field Station of the Imperial College of Science at Slough and the Association is indebted to the Director of the Station and to Professor Brown for their hospitality. Over 70 members and friends attended the meeting.

The attendance at meetings has been on the average 70 per meeting, which is higher than in previous years, and discussion of papers has been active.

During the year, the Association has lost 23 members through resignation, and the Council have, with regret, to record the death of Professor Percy Groom, F.R.S.

The number of new members elected during the year was eight. The Association now numbers 240 ordinary members and 12 honorary members.

During the past year the Association has again enjoyed the privilege of holding its meetings in the Botanical Department of the Imperial College of Science and Technology, and the Council takes this opportunity of recording its grateful thanks on behalf of the Association to the College authorities for this valued hospitality.

The following papers and discussions were brought before the Association during the year 1932.

Feb. 26th. Presidential Address: Dr A. D. Imms, F.R.S.: "Temperature and Humidity in relation to Insect Control."

April 29th. (1) Mr H. S. Schaffer: "The Etiology of Stone Decay."

(2) Dr S. G. Paine: "On the Action of Bacteria in furthering Stone Decay."

(3) Dr Wilesdon: "The Physiology of Stone Decay."

Oct. 28th. (1) Mr R. H. Bunting: "Recent Work on the Deterioration of Stored Products by Fungi."

(2) Mr T. R. Vernon: "The Importance of Fungi in connection with Dairy Produce."

Dec. 9th. (1) Dr A. G. Norman: "Decomposition of Plant Material."

(2) Dr W. G. Campbell: "Decomposition of Wood."

(3) Mr E. R. Speyer: "Investigations in the Biology and Classification of Thysanoptera."

REPORT OF THE HONORARY TREASURER FOR THE YEAR ENDING DECEMBER 31ST, 1932

DURING the year ending December 31st, 1932, subscriptions and entrance fees amounting to £291. 12s. 7d. were received from members. This represents a decrease of £48 as compared with the previous year, and a decrease of over £54 in the last two years. Income from sales of the current volume, reprints and contributions to cost of papers shows a decline of £138. 11s. 1d. as compared with 1931. The fall in income has been met by a reduction of £152. 11s. 7d. in the cost of producing the *Annals*.

The income for the year exceeded the expenditure by £195. 3s. 8d. and, after all obligations have been met, the assets of the Association amount to £1142. 16s. 4d., of which £656. 5s. 0d. is represented by National Savings Certificates. The financial position of the Association is therefore satisfactory. The decline in income from subscriptions and current volume sales is serious. It no doubt reflects the general depression, and, if this is the cause, it may reasonably be hoped that it has reached its maximum. It is, however, very important that members take every opportunity to obtain further subscribers, and also that members pay their subscriptions promptly.

J. HENDERSON SMITH,
Hon. Treasurer.

THE ASSOCIATION OF ECONOMIC BIOLOGISTS

"ANNALS OF APPLIED BIOLOGY" INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED DECEMBER 31st, 1932

Dr

EXPENDITURE

To <i>Annals of Applied Biology</i> :	
Estimated Value of Stock at January 1st, 1932	39 8 6
Cambridge University Press	886 4 2
Copies bought in	57 5 7
	<hr/>
	£982 18 3
	<hr/>
To <i>Annals of Applied Biology</i> , balance brought down	£ s. d.
Printing and Stationery	119 17 0
Postages and Cheque Stamps	5 17 1
Honorariums	4 1 11
Sundry Out-of-Pocket Expenses of Secretaries and Treasurer	10 5 0
Audit Fee Reserve	6 12 9
Balance, being Excess of Income over Expenditure for the year	4 4 0
	<hr/>
	£346 1 5

GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED DECEMBER 31st, 1932

EXPENDITURE

By Members' Subscriptions:	£ s. d.
Arrears	20 0 0
Entrance Fees	3 13 6
Current	267 19 1
	<hr/>
By Interest on National Savings Certificates and Bank Deposit	291 12 7
	<hr/>
	54 8 10

£ s. d.

£ s. d.	£ s. d.
561 12 0	291 12 7
99 8 3	54 8 10
123 10 0	
10 0 0	
1 7 0	
67 4 0	
119 17 0	
<hr/>	
£982 18 3	

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INCOME

By Sales—Current Volume	£ s. d.
By Sales—Back Volumes, Parts and Sets	561 12 0
By Sales of Reprints	99 8 3
By Contributions towards cost of papers, etc.	123 10 0
By Cambridge University Press advertisement	10 0 0
By Estimated Value of Stock at December 31st, 1932	1 7 0
By Balance carried down	67 4 0
	<hr/>
	£982 18 3

BALANCE SHEET, DECEMBER 31st, 1932

LIABILITIES AND SURPLUS

Sundry Creditors:	£ s. d.
Cambridge University Press	157 12 6
Audit Fee Reserve	4 4 0
Out-of-Pocket Expenses of Secretary and Honorarium	10 12 2
	<hr/>
Subscriptions paid in advance	172 8 8
Excess of Assets over Liabilities:	7 17 6
As Balance Sheet of December 31st, 1931	947 12 8
Add Balance of Income and Expenditure Account for 1932	195 3 8
	<hr/>
	£1142 16 4
	<hr/>
	£1323 2 6

ASSETS

Cash:	£ s. d.
At Bank on Current Account	214 12 6
At Bank on Deposit Account	350 0 0
	<hr/>
Debtors for Subscriptions 2 years or less in arrears and considered good	564 12 6
500 National Savings Certificates	35 1 0
Stock of <i>Annals of Applied Biology</i> at estimated value	656 5 0
	<hr/>
	67 4 0

J. HENDERSON SMITH, *Honorary Treasurer.*

We certify that the foregoing Accounts are properly drawn up in accordance with the books, vouchers and documents produced to us, and, in our opinion, the Balance Sheet exhibits a true and correct view of the state of the affairs of the Association.

H. J. COX & CO.

Incorporated Accountants.

Auditors.

THE EFFECT OF SOME CHEMICALS ON
GERMINATION IN COCKSFOOT (*DAC-*
TYLIS GLOMERATA L.)

By H. G. CHIPPINDALE, M.Sc.

(*Welsh Plant Breeding Station, Aberystwyth.*)

DURING the greater part of the nineteenth century the belief was general among practical agriculturists that the germination of seeds could be accelerated by treatment with certain chemicals (*e.g.* oxalic acid and salts of lead), and in recent years interest in the subject has been renewed by the many investigations into the possibility of this occurrence which have been carried out more particularly by continental workers; the researches of Popoff(14, 15) may be cited as showing well-defined positive results. The compounds which this worker found to behave as "stimulants" to germination were exceedingly varied in character, and although an attempt was made to explain the action of all by a single hypothesis, no generalisation has been found tenable by later workers. Among the substances found to stimulate the germination of seeds have been the following: copper sulphate(1), mercuric compounds(6), magnesium chloride(4), lead nitrate(10), manganese and magnesium sulphates(11), sodium chloride(7), oxalic acid(10), ortho-phosphoric acid(5) and the trade preparation Uspulun(17). Other researches, however, have shown no evidence of any stimulation of germination by such compounds(3, 8, 13, 18).

Sampson and Davies(16) have made a critical study of the effect of copper carbonate and Tillantin R (a trade preparation) on the germination of wheat. Their results showed clearly that the apparent stimulation resulting from treatment with these substances was entirely due to their inhibiting fungal infection of the seed and seedling. From an examination of previous literature these authors concluded that in many cases of supposed chemical stimulation the effects were entirely due to the fungicidal action of the compounds employed.

In agricultural practice the results obtained from sowing grass "seeds" in the field are frequently far from satisfactory. The apparent germination is often both slow and meagre, and the resulting plants are entirely ineffective in suppressing the development of seedlings of weeds.

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It is, therefore, peculiarly desirable, should it prove possible, to accelerate or increase by some means the germination of the sown species; the experiments recorded in the present paper were made with the purpose of determining whether any of the substances mentioned above as having been regarded as stimulants to germination could have this effect when applied to the seeds of grasses. No attention was given to the possibility of development subsequent to germination being affected.

METHODS.

The seeds (*i.e.* caryopses with attached pales) used throughout the present work belonged to one strain of indigenous cocksfoot and had been grown and harvested in Britain. The germination of such seeds is considerably less vigorous than is that of commercial samples of the same species, and since cocksfoot is itself characterised by a slow rate of germination relative to that of the other grasses, the results described below can be regarded as applying to seeds markedly deficient in vigour of germination; seeds of this sort have been considered to respond particularly readily to stimulation⁽¹²⁾. All the seeds had been mechanically threshed and, being several months old, were completely after-ripened.

The chemicals were employed as aqueous solutions of three widely different concentrations in each case, the range being determined from preliminary experiments and the results of previous investigators. The seeds were floated on the surface of an excess of the solution in shallow Petri dishes (having, therefore, good aeration) and allowed to soak for 16 hours at 22° C.; subsequently, they were air-dried for about an hour at a temperature of approximately 15° C. before being placed under conditions suitable for germination. The experiments were carried out in duplicate, in one series the seeds being placed on discs of filter paper saturated with distilled water, and in the other being sown in soil contained in large Petri dishes; previous to use the soil was passed through a sieve of 4 mm. mesh and sterilised by autoclaving for half an hour at 125° C. In both series the seeds were subject to the fluctuating temperature of the laboratory, a condition favourable to the germination of cocksfoot seeds and approximating to that obtaining in the field. Control samples of seeds which had been soaked in distilled water (in a similar manner to that already described) and of untreated seeds were allowed to germinate under the same conditions in each experiment.

It should be noticed that by using these substrata, which are free from parasitic organisms, any fungicidal action on the part of the

solutions in which the seeds were soaked is confined to organisms carried by the seeds themselves.

RESULTS.

For convenience of presentation, the germination of the seeds following treatment with the different solutions is given as a percentage of that occurring under the same conditions in seeds which had been soaked in distilled water, but it should be understood that the figures obtained in this way represent the results of several distinct experiments, for each of which the germination of the control seeds was different.

Table I.

Germination on discs of filter paper of cocksfoot seeds after soaking in chemical solutions (expressed as percentages of that occurring in seeds soaked in distilled water).

	After 11 days	After 17 days	After 30 days
Seeds untreated	54	74	97
Seeds soaked in distilled water	100	100	100
" 0.05 % Uspulun solution	89	93	98
" 0.1 % Uspulun solution	66	77	92
" 0.25 % Uspulun solution	52	62	83
" 0.08 % ortho-phosphoric acid solution	87	97	101
" 0.4 % ortho-phosphoric acid solution	75	96	100
" 1.6 % ortho-phosphoric acid solution	53	69	93
" (0.66 % magnesium sulphate + 0.33 % manganese sulphate solution)	65	82	98
" (2 % magnesium sulphate + 1 % manganese sulphate solution)	74	87	99
" (4 % magnesium sulphate + 2 % manganese sulphate solution)	62	75	95
" 1 % magnesium chloride solution	36	—	103
" 3 % magnesium chloride solution	33	—	100
" 6 % magnesium chloride solution	20	—	94
" 0.25 % copper sulphate solution	0	—	8
" 0.5 % copper sulphate solution	0	—	0
" 2.5 % copper sulphate solution	0	—	2
" 0.05 % mercuric chloride solution	10	—	94
" 0.1 % mercuric chloride solution	0.5	—	33
" 0.5 % mercuric chloride solution	0	—	0
" 0.1 % lead nitrate solution	95	94	97
" 0.5 % lead nitrate solution	70	84	91
" 1 % lead nitrate solution	80	100	104
" 0.5 % oxalic acid solution	61	54	64
" 1 % oxalic acid solution	43	55	76
" 2 % oxalic acid solution	46	71	84
" 1 % sodium chloride solution	44	64	80
" 3 % sodium chloride solution	40	82	101
" 6 % sodium chloride solution	31	72	97

The figures in Table I summarise the germination occurring in seeds placed on discs of filter paper. The various elements present in a sample of cocksfoot seed (*i.e.* seeds of different sizes and clusters of seeds attached

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to one rachilla) have been taken at random and each regarded as a single unit; thus no account has been taken of any germination occurring in the other seeds of a panicle-cluster after the germination of one seed has been observed. Only seeds showing both roots and shoots were regarded as germinated. The same procedure of random sampling was adopted in the case of the seeds sown in sterilised soil, the results from which are given in Table II, but in this case germination meant the appearance of a shoot at the surface of the soil and those derived from panicle-clusters could not be distinguished. In both tables each figure is derived from the germination of at least three separate hundreds of seeds.

Table II.

Germination in sterilised soil of cocksfoot seeds after soaking in chemical solutions (expressed as percentages of that occurring in seeds soaked in distilled water).

	After 11 days	After 18 days	After 36 days
Seeds untreated	51	63	64
Seeds soaked in distilled water	100	100	100
" 0.05 % Uspulun solution	92	105	107*
" 0.1 % Uspulun solution	89	98	101
" 0.25 % Uspulun solution	68	91	94
" 0.08 % ortho-phosphoric acid solution	92	96	98
" 0.4 % ortho-phosphoric acid solution	83	89	89
" 1.6 % ortho-phosphoric acid solution	87	87	90
" (0.66 % magnesium sulphate + 0.33 % manganese sulphate solution)	103	102	102
" (2 % magnesium sulphate + 1 % man- ganes sulphate solution)	100	101	102
" (4 % magnesium sulphate + 2 % man- ganes sulphate solution)	95	98	98
" 1 % magnesium chloride solution	90	95	99
" 3 % magnesium chloride solution	80	96	94
" 6 % magnesium chloride solution	43	76	78
" 0.25 % copper sulphate solution	11	85	93
" 0.5 % copper sulphate solution	6	67	98
" 2.5 % copper sulphate solution	0	26	51
" 0.05 % mercuric chloride solution	4	41	69
" 0.1 % mercuric chloride solution	0	8	22
" 0.5 % mercuric chloride solution	0	0	0
" 0.1 % lead nitrate solution	55	72	80
" 0.5 % lead nitrate solution	71	85	90
" 1 % lead nitrate solution	60	78	89
" 0.5 % oxalic acid solution	77	77	78
" 1 % oxalic acid solution	77	88	87
" 2 % oxalic acid solution	64	77	75
" 1 % sodium chloride solution	42	86	99
" 3 % sodium chloride solution	40	67	68
" 6 % sodium chloride solution	36	67	73

* This is the only figure having an excess over the control that is statistically significant.

It is clear from Tables I and II that no evidence at all significant has been obtained either of stimulation or of fungicidal action on the part

of the chemicals employed, and there is little likelihood, therefore, of their being of practical utility. The difference, however, between the germination of seeds which have been soaked in water and that of normal untreated seeds is so great as to suggest that the former treatment may have considerable value in agricultural practice, since not only is germination accelerated in the case of the soaked seeds, but that obtained in them is still greater after 4 weeks than is that of the untreated samples. (Cf. in this connection the work of Tincker(19) and Kinzel(9), of whose results the present are confirmatory.)

The seeds used in the above experiments were sufficiently dry to separate easily and could therefore have been sown in the way usual in the field; it has been found, however, that the beneficial effects of soaking in water are not removed by prolonged drying of the seeds or by drying them to a lower degree of moisture than is found in normal seeds. This is clearly shown in Table III, the figures in which are each representative of the germination of three hundred seeds, the germination medium being sterilised soil.

Table III.

Germination in sterilised soil of cocksfoot seeds following soaking in water and prolonged drying.

	Germination %		
	After 11 days	After 17 days	After 36 days
Seeds air-dried for 2 days	28.3	37.6	40.0
" 3 "	24.6	39.0	46.6
" 5 "	27.6	44.0	46.6
Seeds air-dried for 5 days and for 24 hours over concentrated H_2SO_4 (moisture content=5 %)	26.3	38.6	40.6
Untreated seeds (moisture content=10 %)	9.3	27.6	32.3

Such a degree of independence of any exactitude in the method of treatment increases the applicability of the latter to practical purposes; it should be stated, however, that in soil below the optimum degree of moisture the superior germination of soaked seeds is due in part to their higher moisture content.

The results of an investigation into the way in which this acceleration of germination is brought about have been published elsewhere(2), but there remains to be considered here the possibility that under certain conditions the soaking of seeds in water may depress the subsequent germination.

To obtain data on this point, seeds of cocksfoot which had been soaked in distilled water for 17 hours at 20° C. and air-dried for from

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1 to 7 hours were sown in sterilised soil containing widely different amounts of water, ranging from apparently dry soil (20 per cent. moisture) to soil in a puddled condition (37 per cent. moisture), such as would be quite unworkable in the field. The results are shown in Table IV (the figures in which are again averages of 300 seeds), and give no indication of any inimical results from soaking at any moisture content of the soil. It is noteworthy that many seeds sown in puddled soil remain dormant and do not germinate until after the soil has been dried completely and then remoistened; also that the germination of seeds sown in dry soil and after some time given a satisfactory amount of water is not equal to that occurring in seeds sown directly into moist soil¹.

Table IV.
*Germination of soaked and untreated seeds of cocksfoot
in relation to soil moisture.*

Moisture content of soil %		Germination %			
		After 11 days	After 23 days	After 36 days	After 49 days
20	Soaked seeds (dried 1 hour)	Nil	7.3*	26.0	31
	Untreated seeds	"	5.6*	22.6	27.3
26	Soaked seeds (dried 2 hours)	8.3	25.3*	30.3	34
	Untreated seeds	2.0	17.6*	21.0	23.7
30	Soaked seeds (dried 6½ hours)	17.6	38.3	38.6	39.7
	Untreated seeds	8.3	32.0	32.0	33.3
34	Soaked seeds (dried 5½ hours)	32.0	53.6	54.3	55
	Untreated seeds	10.6	41.3	42.3	44
37	Soaked seeds (dried 4½ hours)	4.0	16.0	21.3	23.7
	Untreated seeds	1.0	5.6	7.3	8.7

* At this time sufficient water added to the soil in these cases to bring the moisture content to 34 %.

It is clear from the above results that the soaking of cocksfoot seeds in water previous to sowing is frequently effective in producing a marked acceleration in germination and that the chances of an inimical action are remote; no objections to its employment in agricultural practice can therefore be seen. There are, however, certain obvious conditions under which no differentiation from the behaviour of untreated seeds would be likely to occur. Such conditions are excessively heavy rain at the time of sowing and the occurrence of low temperatures inhibiting germination during a period in which the seeds are enclosed in damp soil. It is also conceivable that occasionally malting of the partially germinated seeds might take place differentially between soaked and unsoaked seeds, but

¹ It ought perhaps to be stated that the data relating to the behaviour of soaked seeds have been confirmed by experiments on seeds of cocksfoot obtained commercially.

such a possibility is inseparable from any division into two germination periods and the chances of either sort being the one affected are equal.

SUMMARY.

1. Attempts to accelerate the germination of cocksfoot seeds by treating them with solutions of several chemical compounds have been unsuccessful.
2. A pronounced acceleration of germination can be produced by soaking the seeds in water and drying for an indefinite period before sowing.
3. No evidence has been obtained that with any condition of the soil is such treatment undesirable.

The author wishes to thank Professor R. G. Stapledon, to whom he is indebted for criticism and at whose suggestion the work was begun.

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GIBBERELLA SAUBINETII (MONT.) SACC. ON BRITISH CEREALS

III. OCCURRENCE UNDER NATURAL CONDITIONS.

BY F. T. BENNETT, B.Sc., PH.D. (LOND.).

(Adviser in Mycology and Agricultural Botany, Armstrong
College, University of Durham, Newcastle-upon-Tyne.)

(With 1 Text-figure.)

THE occurrence of the perithecial stage of *Gibberella Saubinetii* on cereals is of interest to the agriculturist as well as the mycologist, because grain so affected has proved toxic to pigs and other domestic animals both on the Continent and in the U.S.A. Some affected feeding barley imported into England and Germany from the U.S.A. caused losses to pig-feeders during the winter of 1928-9. The writer has, in previous papers (1, 2), described this fungus in its conidial stage (*Fusarium graminearum*) from cereal crops, and its perithecial stage as produced under artificial conditions. It was there stated that the average seasonal climatic conditions did not suffice for the development of perithecia in the field. During 1932, however, this stage was found on the ears of Little Joss wheat at Cockle Park, Northumberland, in an isolated plot sown for rust observations; this is the first record of its occurrence in England under natural conditions¹.

The perithecia at the end of September were visible to the eye as black dots or small patches associated with a salmon-coloured conidial growth approaching a "mucous mould" form, along the edges and sides of the glumes. Some perithecia were mature, asci and ascospores being discharged when transferred to water. Some immature ones developed perfect ascospores by the fourth day in a moist chamber at room temperature, and these, probably, would have matured outdoors also. Still other perithecia were not mature after one month in the moist chamber, and these, doubtless, would remain dormant until the spring (2), p. 164).

¹ The fact that the writer has studied the cultural and pathogenic characters of this fungus renders desirable a definite statement that this occurrence was strictly fortuitous; no experimental work has been done outdoors since 1929, and at no time within about twenty miles of the farm named.

In 1929 artificially inoculated wheat and barley did not bear perithecia under outdoor conditions; in 1932 perithecia occurred naturally. Since the rainfall of the summer months of these years did not differ greatly (6.91 and 7.71 in. respectively), and the precipitation was distributed fairly uniformly throughout the period, the requisite degree of atmospheric humidity probably existed in both seasons; the determining factor would then be the temperature. The temperature range for the two seasons is shown in Fig. 1.

The graph does not, however, indicate temperatures in the open, which, in "heat wave" periods in 1932, were unusually high; it is

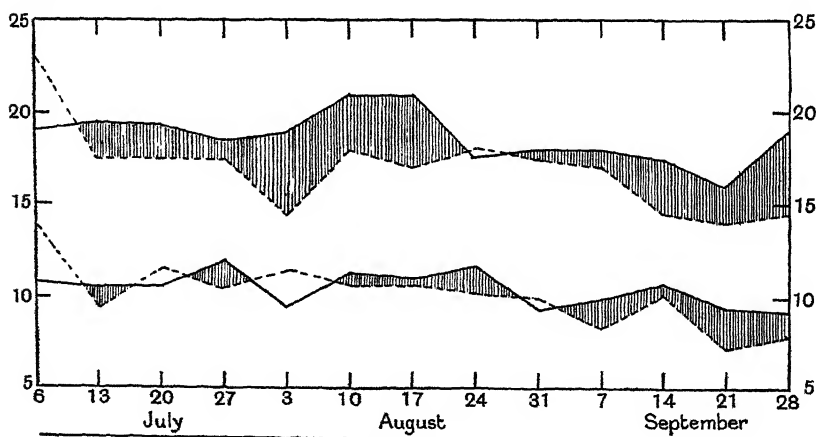


Fig. 1. Average weekly maximum and minimum shade temperatures. — 1932, at Cockle Park, altitude 326 ft., distance from sea 6 miles. - - - - - 1929, at Newcastle, altitude 100 ft., distance from river 1 mile, from sea 8 miles. ||||| Periods during which the mean temperature was higher in 1932.

believed that this factor is of importance as well as mean temperature. The mean temperature, however, was of higher maximum for about ten weeks, and higher minimum for seven weeks out of the twelve in 1932 than in 1929. These joint conditions of higher mean temperature and occasional very hot days sufficed for the production and maturation of perithecia. There can be little doubt that similar conditions of temperature and humidity occur in other parts of this country, and that, in seasons like 1932, the perfect stage of *G. Saubinetii* will not be uncommon on cereals.

Note on cultures. It has been stated (11, p. 52) that single ascospores may give rise to one of two types of growth, which may be called the

Fusarium and ascigerous types respectively. Investigation of the conditions determining the type shows the *Fusarium* product to be the common and normal one. It was produced from ascospores from the following sources:

(1) Naturally infected wheat grains producing perithecia during germination tests (Seed Testing Station, 1930).

(2) Naturally infected wheat ears producing perithecia in the field (Cockle Park, 1932).

(3) Wheat and barley ears artificially inoculated during the growing season, and perithecia matured in moist chambers (*a*) immediately afterwards, (*b*) after storage indoors until April, (*c*) after storage indoors until June, (*d*) after wintering outdoors until April.

(4) Barley grains from ears inoculated during growth, stored indoors until June, then heated up to 3 min. at 100° C., perithecia being developed afterwards in moist chambers.

(5) Pure cultures on cooked wheat grains, perithecia formed thereon (*a*) on sterile soil, (*b*) after submission to low temperature (– 20° to – 1° C.) for periods up to 30 days.

(6) On bases and stem internodes of wheat and barley seedlings grown in contaminated soil and from contaminated seed, and perithecia developed thereon in moist chambers in July; test cultures made (*a*) immediately, and (*b*) after storing 3 months.

(7) In pure cultures of the *Fusarium* stage yielding perithecia on hard oat agar.

None of these diverse conditions, imposed on the fungus before and after the formation of perithecia, modified the type of growth from single ascospores. It should be mentioned that on salts-glycerine agar (1, p. 48) the growth from ascospores always more nearly resembles the ascigerous type than the *Fusarium* type as obtained from single conidia. The medium is, therefore, useful when investigating perithecia which are associated with the conidial stage.

The ascigerous type of growth has not been reproduced since its first occurrence. It then arose from single ascospores and from immature asci without definite ascospores, both being from perithecia produced on naturally infected wheat grains. These grains, placed in moist chambers in October, lay in warm and cold rooms (0–18° C.), sometimes moistened and at times dried out, at irregular intervals until April, at which time the crust of perithecia on the grains was overgrown with mycelium. It remains, only, to emphasise the fact that, after abnormal conditions,

this type of growth may appear, but it is not the normal type of growth from ascospores.

SUMMARY.

Gibberella Saubinetii (Mont.) Sacc. occurred in its perfect stage under field conditions on wheat ears in 1932. The meteorological conditions are compared with those of a season when perithecia were not developed outdoors.

Single ascospore growths in culture are normally of the *Fusarium* type, with a characteristic modification on salts-glycerine agar. The ascigerous type of growth may arise from ascospores developed under abnormal conditions.

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PINK ROT OF THE POTATO

BY H. CAIRNS, B.Sc., M.AGR.

AND

A. E. MUSKETT, M.Sc., A.R.C.S.

(*Ministry of Agriculture and Queen's University, Belfast.*)

(With Plate XXII and 1 Text-figure.)

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INTRODUCTION.

IN 1912 while working at Clifden in the west of Ireland Pethybridge⁽¹²⁾ drew attention to a peculiar *Phytophthora* soft rot of the potato tuber; for this very characteristic disease he proposed the term "Pink Rot," the name by which the disease is now generally recognised. It seems very probable that this type of tuber rot was recognised by growers in particular districts before Pethybridge demonstrated its actual nature; it has been found on several occasions that the general terms "water slain," "water rot," and "soft rot," are used by growers as synonyms for "pink rot," while the disease is also occasionally attributed to frost injury. In 1913-14 Pethybridge^(10, 11) published his observations on the nature and importance of the disease. In 1915 he wrote⁽¹⁴⁾ concerning the finding of the disease in Holland during 1914, but apart from this record, reports during the succeeding five years appear to be confined to its occurrence in Ireland. Further records of its occurrence from 1919 until the time of writing are given below.

Scotland: first recorded by Cotton⁽³⁾ in 1919: McIntosh⁽⁷⁾ records in 1927 its having been found in Berwickshire and Midlothian.

England and Wales: first recorded by Cotton⁽⁴⁾ in 1921 from six neighbouring localities in Shropshire; also from Hertfordshire and in potatoes received from Scotland.

English records given by Pethybridge^(18, 19, 20) are:

1922: Shropshire and Berkshire.

1923: Shropshire, Kent and Lancashire.

1924: Kent and Durham.

1926: Yorkshire (two centres), Lancashire, Shropshire and Monmouthshire.

1927: Yorkshire.

1928-31: Lincolnshire (two records), Yorkshire (seven records; two attacks were severe), Staffordshire (two records), Flintshire, Leicestershire, Cambridgeshire (attack severe) and in Cheshire (attack severe).

Holland: reported (1915) by Pethybridge⁽¹⁴⁾: isolated by van Luijk in 1914. Reported (1922) by van Poeteren⁽²⁵⁾.

Bulgaria: reported (1927) by Atanasoff and Kovačevski⁽²⁾ in potatoes grown near Sofia.

United States of America: reported (1923) by Drechsler⁽⁶⁾ in Oklahoma and Kentucky.

Dutch East Indies: reported (1923) by Paravicini⁽⁹⁾ in crop exported from Java. Reported (1923) by van Hall⁽²⁴⁾ in crop near Preangar, Java.

During the period 1922-7 the Ministry of Agriculture for Northern Ireland received various reports relating to the occurrence of this disease in the area under its jurisdiction. These reports indicated the desirability of an enquiry into the occurrence and general importance of the disease in this area. Accordingly, investigations were commenced in 1927 and an account of the work which has been carried out is given in this paper.

SURVEY OF THE OCCURRENCE OF THE DISEASE IN NORTHERN IRELAND.

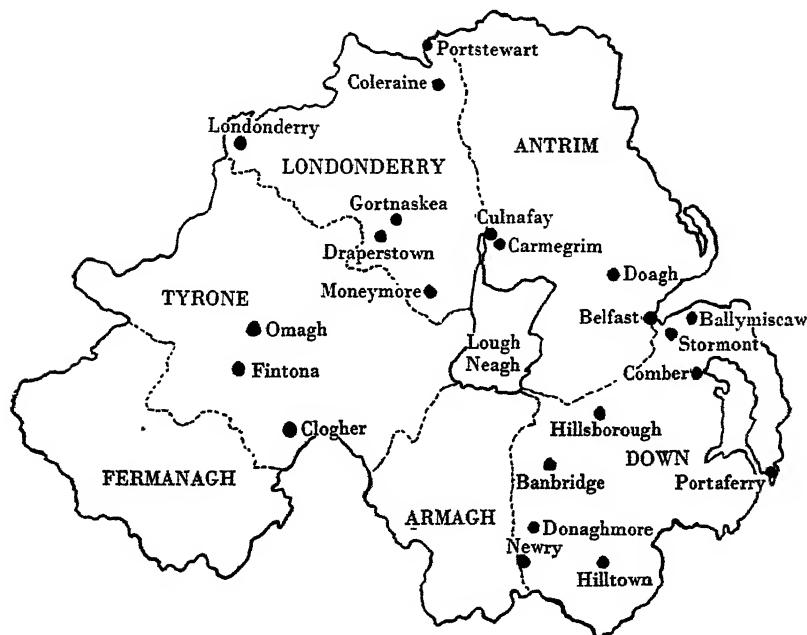
During 1929 the Instructors in Agriculture and the Potato Inspectors in the Province were invited to report any suspected cases of the disease which they might encounter and, at the same time, to submit specimen diseased tubers together with details of soil, manuring, crop rotation, etc. This resulted in thirteen confirmed cases of the disease being recorded, the parasite being isolated in each case. This survey was continued in 1930, a further twelve cases being recorded, in most of which no attempt was made to isolate the parasite. Altogether the disease has been

recorded in thirty-one localities during this work; only a few of these occurrences were reported as severe.

The distribution of the disease as indicated by these records is shown in the attached map of Northern Ireland and the complete list of records with relevant notes is given below:

County Antrim: Four centres:

Belfast: Diseased tubers found in a consignment of unknown origin.



Map of Northern Ireland showing centres where Pink Rot of the potato has been recorded (1927-32).

Doagh: Percentages of tubers affected apparently not more than 1-2 per cent. in different varieties.

Carmegrim: Percentages of tubers affected apparently not more than 1-2 per cent. in different varieties.

Culnafay: In this case in 1929 approximately half of the crop from several acres was destroyed during storage. Similar heavy losses of crop had been experienced by the grower in at least three previous seasons. The disease has reappeared in each subsequent season but has not been the cause of further appreciable loss, except in the present (1932) season, in which at least 10 per cent. of the crop from about 0.5 acre

of the variety Field Marshal was found to be affected on raising the crop.

County Londonderry. Eleven centres:

Moneymore: Four cases of slight attack recorded in 1929 and a fifth in 1931.

Draperstown: One case of slight attack recorded in 1929 and a second in 1930; in the first case the grower had observed a much more severe attack in the previous season.

Gortnaskea: One severe attack was recorded.

Portstewart: One severe attack recorded; 50 per cent. of the crop reported as affected.

Coleraine: One slight attack recorded.

Londonderry: One slight attack recorded: disease reported present in previous season.

County Tyrone. Three centres:

Omagh: One severe attack recorded: almost entire crop destroyed.

Clogher: One slight attack recorded.

Fintona: One severe attack recorded: at least 33 per cent. of the variety Arran Chief affected.

County Down. Thirteen centres:

Ballymiscaw: One slight attack recorded.

Portaferry: Disease prevalent in 1929 and 1930.

Comber: One slight attack recorded: less than 1 per cent. of the tubers affected on raising crop; this increased to about 5 per cent. during storage in pits for a period of five months.

Hillsborough: One very slight attack recorded: about ten tubers affected in crop from 0.5 acre.

Newry: Four slight attacks recorded.

Hilltown: One slight attack recorded: disease noted present in previous season.

Donaghmore: One attack recorded: disease stated to be prevalent.

Banbridge: One slight attack recorded.

Stormont: One slight attack recorded: a few affected plants found in experimental plots.

The results of this survey indicate that:

(a) In most cases in the field the percentage of the crop affected is small, and usually so small that the presence of the disease escapes detection.

(b) The disease may be the cause of heavy loss of crop in the field and especially during storage.

So far no records of the occurrence of the disease have been received from the counties of Armagh and Fermanagh.

OBSERVATIONS ON THE CAUSE OF PINK ROT. ✓

Since Pethybridge first demonstrated that pink rot of the potato may be due to *Phytophthora erythroseptica* Pethyb. it seems to have been generally assumed that in the field the disease is due to this species alone. Much evidence has, however, been obtained which shows that the same symptoms may be produced through attack by other species of *Phytophthora*. Drechsler⁽⁶⁾ recorded a species, subsequently named *P. drechsleri* by Tucker, found causing a pink rot of potatoes in the United States. Rosenbaum⁽²²⁾ found that *P. arceae* (Colem.) Pethyb. could cause the disease and suggested that this species and *P. erythroseptica* are probably identical. More recently Drechsler⁽⁶⁾ and Tucker⁽²³⁾ in particular have drawn attention to the large number of *Phytophthora* spp. which will cause pink rot of the potato tuber.

Westerdijk and van Luijk⁽²⁶⁾ referred to *P. erythroseptica* attacking *Atropa belladonna* in Holland, while Alcock⁽¹⁾ recorded a variety of the species (*P. erythroseptica* var. *atropae*) which causes a virulent stem rot of *A. belladonna*.

In view of the reported existence of atypical forms of the pathogene, and of the fact that some variation occurred in the symptoms of the disease shown by certain tubers submitted for examination, it was considered desirable to compare the isolations of the pathogene from different districts in Northern Ireland with as many isolations from other sources as possible.

This comparison of isolations of *Phytophthora* from potatoes affected with pink rot was carried out on the following bases: (a) growth and formation of reproductive organs in artificial media, (b) size and form of oospores, (c) virulence in artificially inoculated tubers of the variety Arran Victory¹. The different isolations used and the results obtained are given in Table I. (Unfortunately cultures of the isolations from the

¹ Except where otherwise stated, the technique of tuber inoculation and incubation was as follows: Tubers were sterilised in a weak solution of formalin or mercuric chloride. Inoculation was effected through a slit cut in the tuber at the "heel" end. Incubation was carried out in shallow wooden boxes (2 ft. square x 6 in. deep) fitted with glass covers and lined with moist absorbent paper to provide a humid atmosphere and at laboratory temperatures varying between 15 and 21° C. The time necessary for the first tuber in an experiment to become completely affected with the disease (11 days) was taken as a time limit for incubation. At the end of the experiment each tuber was cut in halves along its longi-

pink rot occurrences in Holland, Java, Bulgaria, and U.S.A. were not available.) The results of this comparison indicate that, both in its morphological characters and in its virulence to potato tubers, very little variation occurs within the species *P. erythroseptica*. *P. erythroseptica* var. *atropae* Alcock caused pink rot; this was confirmed by a "check" culture of this variety. The isolation "B" from Culnafay, Northern Ireland (No. 15, Table I), which has since been identified as *P. megasperma*¹, also caused typical pink rot, but differed from *P. erythroseptica* by the preponderance of paragynous antheridia and the production of very large oospores. This is the first record of pink rot being caused by *P. megasperma*, and is, moreover, the first record of the occurrence of this species in Britain.

Because of the recorded pathogenicity of other *Phytophthora* spp. to the potato tuber this investigation was extended to include cultures of other species of *Phytophthora* represented in Britain (omitting *P. infestans*). The cultures used and the results obtained are given in Table II.

The main point brought out by these results is that typical pink rot was caused in potato tubers by *P. cryptogea* Pethybr. and Laff., *P. megasperma* Drechsler (isolated from potato affected with pink rot, Northern Ireland), *P. cactorum* (Leb. Cohn.) Schroet. (isolated from strawberry fruits, N. Ireland) and *P. erythroseptica* var. *atropae* Alcock as well as by *P. erythroseptica* Pethybr. The species of *Pythium* isolated by Pethybridge and Smith (21) from potatoes affected with "watery wound rot," a disease similar to "leak," produced a very soft and watery form of rot. Definite pinking symptoms were observed when diseased tubers were cut and exposed to the air, but this type of rot may easily be distinguished from pink rot by its soft and wet nature.

P. porri Foister, *P. syringae* Kleb., *P. richardiae* Buis., and four isolations of *P. cactorum* (L. and C.) Schroet. from apple, pear and strawberry fruits, did not cause the disease.

Some species are incipient in their virulence, as indicated by the three cultures marked * in Table II; in each of these cases a few of the inoculated tubers became affected with pink rot to a slight extent. The virulence of these three cultures to different varieties of potato tubers was also investigated (April, 1931). Five varieties were used and in these it was found that the three cultures were more virulent in the varieties *tudinal axis*: after exposure to the air for 2-3 hours the diseased area had become strongly coloured and the extent to which the disease had progressed was then easily measured as a radius from the point of inoculation. The variety of the potato used was Arran Victory.

¹ Thanks are due to Mr Ashby of the Imperial Mycological Institute, to whom the culture was submitted and who kindly identified the species.

Table I.

Culture and origin	Growth and reproduction in artificial media (oat, potato mush and Coons' agars)	Average diameter of approximately 100 oospores			Variation in size of oospores	Type of rot produced in potato tubers (var. Arran Victory)	Extent of progress of rot in tubers at laboratory temperatures after 12 days cm.
		Potato mush agar	Quaker Oat agar	No oogonia†			
1. <i>P. erythrosepica</i> , forwarded by Pethybridge to C.B.S.* in 1923	(a) All cultures produced aerial hyphae profusely and to about the same extent on oat and potato agars; production on Coons' agar was very scanty	29.8 μ	30.4 μ	No oogonia†	—	Typical pink rot	4.3
2. <i>P. erythrosepica</i> , forwarded by Pethybridge to C.B.S. in 1929	(b) All cultures grew at approximately the same rate	32.8	31.7		10-40 μ	Typical pink rot	4.2
3. <i>P. erythrosepica</i> , Yorkshire, Eng- land (Millard)	(c) No sporangia have ever been found in any culture on solid media	31.1	31.2		22-40	Typical pink rot	4.6
4. <i>P. erythrosepica</i> , Omagh, N. Ireland	(d) Oospores were pro- duced freely by all cul- tures except No. 1 on oat and potato agars.	32.0	32.2		27-39	Typical pink rot	5.5
5. <i>P. erythrosepica</i> , Draperstown, N. Ireland	None were produced on Coons' medium	31.5	32.0		24-40	Typical pink rot	4.9
6. <i>P. erythrosepica</i> , Moneymore, N. Ireland		32.0	32.6		22-40	Typical pink rot	5.3
7. <i>P. erythrosepica</i> , Oranmore, N. Ireland		31.0	31.7		19-43	Typical pink rot	4.8
8. <i>P. erythrosepica</i> , Hillsborough, N. Ireland		30.5	31.5		20-40	Typical pink rot	5.3
9. <i>P. erythrosepica</i> , Portaferry, N. Ireland		30.6	31.2		25-40	Typical pink rot	5.8
10. <i>P. erythrosepica</i> , Portstewart, N. Ireland		31.0	31.2		24-39	Typical pink rot	4.2
11. <i>P. erythrosepica</i> , Doagh, N. Ireland		32.0	31.0		24-37	Typical pink rot	5.0
12. <i>P. erythrosepica</i> , Carnegrim, N. Ireland		33.9	31.5		22-39	Typical pink rot	5.3
13. <i>P. erythrosepica</i> , Culnafay A. N. Ireland		32.4	33.4		24-40	Typical pink rot	5.3
14. <i>P. erythrosepica</i> var. <i>atropace</i> , Scotland (Alcock)		41.6	41.0		19-48	Typical pink rot	4.9
15. <i>Phytophthora</i> species, Culnafay B, N. Ireland					21-48	Typical pink rot	4.3

* C.B.S. = Central Bureau voor Schimmelcultures, Baarn, Holland.

† Dr Westerdijk reported that this culture had ceased to produce oogonia.

Table II.

Caused "pink rot" in inoculated tubers			Failed to cause "pink rot" in inoculated tubers		
Culture used	Tubers inoculated: 30 : 30	Type of rot produced Typical pink rot	Average extent of progress of disease in tubers, cm.	Culture used	Tubers inoculated
<i>P. erythroseptica</i> (isolated from potato, N. Ireland, 1927)			4-2	<i>P. richardiae</i> (supplied by C.B.S.)	10
<i>P. erythroseptica</i> (isolated from potato, N. Ireland, 1930)	15 : 15	do.	4-3	<i>P. parvistica</i> (isolated from tomato, England (Bewley))	20
<i>P. erythroseptica</i> (supplied by Pethybridge to C.B.S., 1929)	10 : 10	do.	4-2	<i>P. parvistica</i> (isolated from tomato, Leonian; supplied by C.B.S.)	15
<i>P. erythroseptica</i> (isolated from potato, Yorkshire, Millard)	10 : 10	do.	4-1	<i>P. syringae</i> (supplied by C.B.S.)	15
<i>P. erythroseptica</i> var. <i>atrocae</i> (isolated from <i>Atropa belladonna</i> , Scotland, Alcock)	30 : 30	do.	4-2	<i>P. cactorum</i> (isolated from apple, N. Ireland, 1929)	15
<i>P. cryptogea</i> (supplied by C.B.S.)	20 : 20	do.	3-2	<i>P. cactorum</i> (isolated from strawberry, Derry, N. Ireland, 1931)	5
<i>P. cryptogea</i> (isolated from tomato, N. Ireland)	10 : 10	do.	2-9	<i>P. cactorum</i> (isolated from apple; U.S.A., Leonian; supplied by C.B.S.)	25
<i>P. cactorum</i> (isolated from strawberry, Armagh, N. Ireland, 1931)	25 : 18	do.	2-9	<i>P. cactorum</i> (isolated from apple, N. Ireland, 1932)	10
<i>P. megalosperma</i> (isolated from potato, N. Ireland, 1929)	30 : 13	do.	3-6	<i>P. cactorum</i> (isolated from pear, N. Ireland, 1932)	10
<i>Pythium</i> sp. (isolated from potatoes affected with "Leak," Pethybridge)	10 : 10	Very soft form of pink rot	4-2	<i>P. porri</i> (isolated from leek, Scotland, Foister)	10

* In each of these cases one or more of the tubers became affected to a slight extent with an incipient form of pink rot.

Remarks
A dry rot was produced to the extent of a few millimetres from wound

do.*

do.*

No pathogenic effect

do.

do.

May Queen and Arran Victory than in Kerr's Pink, Donard, and Golden Wonder; in no case, however, was a vigorous form of pink rot produced such as that in the control tubers inoculated with *P. erythroseptica*.

OBSERVATIONS ON THE NATURE OF THE DISEASE.

The importance of pink rot as a storage disease.

That pink rot is of some importance in the field is generally known, but it does not seem to be generally realised that during storage, under certain conditions, it may be a far more serious disease. During the course of this work several cases have been recorded in which pink rot has destroyed 50 per cent. or more of the tubers stored in clamps. The results obtained from the following experiment give some indication of the extent to which rotting may occur under different conditions of clamp storage.

In 1931 a plot of approximately 0.05 acre, which in the previous season had borne crops affected with pink rot, was planted with tubers of the variety Arran Victory. On harvesting it was found that about 1 per cent. of the tubers were affected with the disease; these were discarded and the healthy tubers stored temporarily in a clamp for 14 days. At the end of this period the crop was again examined and any tubers which had become noticeably affected during the interval were removed. The healthy tubers were now divided into four lots, each of which was stored (5. x. 32) in a clamp as shown in Table III.

Table III.

Clamp	Method of covering	% tubers affected with pink rot after 19 weeks' storage
1	Tubers covered with soil only; soil layer 6-9 in. deep	29.0
2	Tubers covered with layer of herbage 3-6 in. deep plus soil layer 2-4 in. deep	3.0
3	Tubers covered with layer of herbage 6-12 in. deep	3.5
4	Tubers covered as in (2) but with four "ventilators" * through soil layer near the base and one "ventilator" at the apex of the clamp	3.0

* Round aperture through soil layer; 4-6 in. in diameter and loosely filled with herbage.

The clamps remained untouched for a period of 19 weeks, after which they were opened and the tubers graded into (a) healthy, (b) affected with pink rot. When the clamps were opened it was evident that the tubers in clamp 1 had been subject to much moister conditions than those in the other three. The diseased tubers in the clamps 2, 3, and 4 occurred in each case at the base of the clamp. Both in the field and in the clamps the quantity of tubers affected with rots other than pink

rot was negligible. A similar experiment carried out (24.x.32-8.iii.33) with the same variety gave confirmatory results. Each clamp contained 224 lb. tubers. Of these, it was found that, where conditions were very moist, 27.0 per cent. were affected with the disease. In the other clamps, in which conditions were relatively dry, the percentages of affected tubers varied from 1.0 per cent. to 2.0 per cent.

These results indicate that the occurrence of pink rot during clamp storage may be largely controlled by ensuring efficient aeration of the clamp. Reference to Table V, column 7, shows that in a varietal susceptibility experiment carried out in 1930 an average of 26 per cent. of the tubers of different varieties became infected with pink rot while being stored in clamps covered with soil.

The disease and the growing plant.

The term "pink rot" refers to the pathological condition brought about in the potato tuber, and in general practice the disease is still regarded as essentially a disease of the tubers. Pethybridge^(11, 14) showed that this need not necessarily be the case and that the pathogene may give rise to a systemic disease of the growing plant for which he suggested the name "pink rot wilt." This aspect of the disease was investigated by making the following experiments.

I. Three drills in a field were each planted with twenty-five healthy, strongly budded tubers. Each tuber in the first drill was inoculated with *P. erythroseptica* at the time of planting by means of an incision at the "heel" end. In the second drill pieces of tuber affected with the disease were placed in close proximity to each "set." The third drill was used as a control.

All "sets" in the second and control drills gave rise to healthy plants and, on raising the crop, no tubers were found to be infected with the disease. In the case of the first drill eleven of the "sets" produced no plants thus causing "misses," while the remainder produced weak, spindly plants much smaller than the controls. The progeny from these plants were free from the disease.

II. In the winter and spring of 1929-30 two series of pot experiments were carried out with the object of investigating the relationship between the time of infection of the tuber with *P. erythroseptica* and its effect upon the resultant plant. Selected, healthy tubers of the variety British Queen were used, each having a single, strong, main bud. Each tuber was planted close to the surface of the soil in a 10 in. pot and kept in a cool greenhouse. All inoculated¹ tubers were examined about 14 days

¹ The technique of inoculation was to expose the upper surface of the tuber and sterilise with a cloth soaked in alcohol. The inoculation wound was covered with sterilised grease-proof paper and vaseline. The tuber was then recovered with soil.

after inoculation, when it was found that in each case typical pink rot had been produced. The experiments were continued until the control plants had reached maturity.

These two series of experiments are considered together, and the details of inoculation together with the results obtained are shown in Table IV. From this table it will be seen that all control tubers (*a*) gave rise to healthy plants. "Misses" were produced in the case of a number of the tubers which were inoculated at the time of planting (*b*, *c*, and *d*). The proportion of "misses" was greatest where very slightly budded tubers were used (*b*), which indicates a direct relation between the time of infection and that which must elapse before establishment of the daughter plant. In many cases where plants were produced (*b*, *c*, and *d*) they sooner or later developed symptoms of "pink rot wilt" and died prematurely. Infection of the tuber had no effect on the resultant plant when it was well established (*f*).

Table IV

Time of inoculation	Results
(<i>a</i>) Control. 15 <i>strongly</i> budded tubers: not inoculated	All tubers gave rise to healthy plants which reached maturity
(<i>b</i>) 10 <i>very slightly</i> budded tubers: inoculated at "heel" end at time of planting	1 tuber produced very weakly growing plant: reached maturity 1 tuber produced very weakly growing plant: developed "pink rot wilt," and died before maturity 8 tubers failed to produce plants
(<i>c</i>) 15 <i>strongly</i> budded tubers: inoculated at "heel" end at time of planting	8 tubers produced weakly growing plants: reached maturity 6 tubers produced weakly growing plants: developed "pink rot wilt" and died before maturity 1 tuber failed to produce a plant
(<i>d</i>) 10 <i>strongly</i> budded tubers: inoculated at insertion of main bud ("rose end") at time of planting	7 tubers produced weakly growing plants: reached maturity 1 tuber produced weakly growing plant: developed "pink rot wilt" and died before maturity 2 tubers failed to produce plants
(<i>e</i>) 10 <i>strongly</i> budded tubers: inoculated when main shoots had grown about 2 in. above soil level	All tubers gave rise to plants which reached maturity: slightly smaller than controls
(<i>f</i>) 5 <i>strongly</i> budded tubers: inoculated when main shoots had grown about 6 in. above soil level	All tubers gave rise to plants which reached maturity: indistinguishable from controls

Photographs of typical plants from these experiments are shown in Plate XXII.

The question of the susceptibility of the growing plant to pink rot wilt after it has become established, and by other means of infection than

through the seed tuber, is under investigation. In 1915 Pethybridge⁽¹⁴⁾ recorded that this aspect of the disease could be produced by wound inoculation of the stem just below soil level.

The results obtained from these experiments in general indicate that:

(1) If a tuber becomes affected with pink rot at a stage where the plant cannot quickly become established, it is destroyed before the establishment of the plant and so a "miss" occurs in the crop.

(2) If a tuber becomes affected after it has given rise to a strongly established plant, the plant is able to resist attack by the fungus and is unaffected.

(3) A weakly established plant may become affected by the disease in the form of "pink rot wilt," the severity of the attack varying according to the interaction of the various factors which govern the progress of the disease in the mother tuber and establishment of the daughter plant.

The coloration changes in tubers affected with pink rot.

That certain colour changes occur in the exposed tissues of a wounded potato tuber is well known. This phenomenon takes place during the course of a few hours, the coloration passing through a faint red to a faint brown tint. The colour phenomenon associated with parasitism by various species of *Phytophthora* (pink rot), *Pythium* sp. and also, under certain conditions, by certain bacteria is apparently of a similar but very much more intense nature. Symptoms more or less like those of pink rot are also produced where, under conditions of very poor aeration such as prolonged submergence under water, potato tubers may become subject to a form of "water rot"; such tubers when cut and exposed to the air develop a pink-brown coloration in the exposed tissue.

That this coloration occurring in tubers affected with pink rot is a function of the tuber alone, is shown by the effects resulting from exposure of freshly wounded healthy tubers to the vapour of such lethal substances as chloroform or toluol. This can be demonstrated as follows. A healthy tuber is cut in two and one of the portions exposed for about 30–60 min. to the vapour of chloroform in a closed dish. A tuber affected with pink rot is now cut in two and both parts of each tuber are left for a time exposed to the air. It will be found that intense coloration occurs in the cut surface of the tuber exposed to the lethal vapour and that this is very similar to that occurring in the tuber affected with pink rot. No appreciable coloration occurs in the other part of the healthy tuber¹.

¹ In the laboratory the application of this principle provides a very convenient means for demonstrating the colour changes which occur in tubers affected with pink rot.

Onslow⁽⁸⁾ and others have shown that, on the death of the tuber cell, which may be brought about by injury or by poison such as chloroform or by toxins secreted by parasites, autolysis occurs and an oxidisable substance is released. Exposure to air brings about the oxidation of this substance, which results in the production of the coloured substance whereby the characteristic colour changes observed in a diseased tuber may be explained.

VARIETAL SUSCEPTIBILITY OF THE POTATO TO PINK ROT.

Pethybridge^(16, 17, 18, 20) has recorded the following varieties of the potato as being more or less susceptible to pink rot: K. Seedling, Champion II, Northern Invincible, Langworthy, Golden Wonder, Kerr's Pink, Dominion, Provost, Burnhouse Beauty, Great Scot, King Edward, Eclipse, Duke of York, Big Ben, Sharpe's Express, Red King, and Majestic. Cotton⁽⁴⁾ records the varieties Majestic, Great Scot and King Edward. During the course of this work the disease has been found in Northern Ireland attacking the varieties Up-to-Date, Arran Consul, Arran Victory, Arran Chief, Arran Banner, Field Marshal, Kerr's Pink, and a number of others whose identity was not definitely ascertained. Of these outbreaks nine have been recorded in Arran Victory, which is undoubtedly a fairly susceptible variety. It would appear, however, that the frequent recognition of the disease in the tubers of this and other coloured varieties is at least partly due to the more striking external symptoms caused by the breakdown of the pigment and consequent discoloration of the skin resultant upon attack.

In order to obtain some measure of the relative susceptibilities of potato varieties grown in Northern Ireland, comparative infection experiments were conducted on the following lines.

(a) *Effect of artificial inoculation of tubers with P. erythroseptica.*

Five healthy tubers of each of thirty varieties were inoculated as described on p. 385. The varieties used and the average rates of progress of the disease in the tubers are shown in Table V, columns 1 and 2. This experiment was repeated twice, and as the results showed fair agreement on each occasion they are recorded collectively.

(b) *Effect of storage of healthy tubers in infected soil.*

Ten healthy tubers of each of twenty-five varieties were stored for 8 weeks in 10 in. flower pots filled with moist soil which had borne an infected crop in the previous season. One pot was used for each variety. In addition to the natural infection of the soil, layers of pure cultures of

P. erythroseptica were placed at some distance above and below the tubers. During the experiment each pot was placed in a saucer, which was kept filled with water, and was covered with sacking in order to prevent undue evaporation. The results from this experiment are shown in Table V, column 3.

Table V.

Variety	Average extent of rot in 15 inoculated tubers cm.	Number of tubers affected with rot during 8 weeks in infected soil (10 tubers per pot)	Percentage of crop affected with rot at time of harvesting crop			Percentage of 1930 crop affected during storage (4 months) in clamps
			1930 %	1931 %	1932 %	
1. Abundance	—	4	20.0	—	—	—
2. Alpha	4.4	6	4.3	4.0	—	29.0
3. America	—	6	—	—	—	—
4. Arran Banner	5.4	1	3.0	0.0	0.5	36.0
5. Arran Chief	—	—	—	—	1.2	—
6. Arran Crest	5.3	—	—	10.0	2.1	—
7. Arran Comrade	4.6	0	2.0	8.0	1.9	36.0
8. Arran Consul	5.5	8	3.5	5.0	4.5	41.0
9. Arran Pilot	5.5	—	—	0.0	2.9	—
10. Arran Victory	4.6	0	2.5	6.0	0.0	26.0
11. Ballydoon	4.7	—	—	—	1.9	—
12. Ben Lomond	5.6	—	—	0.0	—	—
13. British Queen	5.3	4	7.0	2.0	5.9	28.0
14. Catriona	—	5	—	29.0	7.0	—
15. Champion	4.4	—	—	3.0	0.7	—
16. Di Vernon	—	—	—	11.0	—	—
17. Donard	5.2	—	—	13.0	6.2	—
18. Doon Star	4.5	—	—	—	—	—
19. Duke of Perth	5.1	—	—	—	—	—
20. Duke of York	—	6	25.0	—	—	—
21. Dunaverny	6.1	—	—	—	2.7	—
22. Early Templar	5.4	—	—	—	—	—
23. Edzell Blue	—	2	7.0	0.0	—	59.0
24. Epicure	5.9	5	3.0	—	3.5	37.0
25. Field Marshal	—	2	0.0	—	0.4	13.4
26. Field Marshal*	—	0	0.0	3.0	—	41.0
27. Golden Wonder	—	2	3.0	4.0	1.1	24.0
28. Great Soot	5.2	5	1.5	2.0	0.7	25.0
29. Herald	5.6	—	—	—	—	—
30. Incomer	4.3	1	0.0	—	—	12.0
31. Kerr's Pink	5.3	3	0.0	1.0	4.5	3.0
32. King Edward	5.8	6	1.5	3.0	0.5	12.0
33. Lochar	4.6	2	1.5	—	—	20.0
34. Majestic	5.7	3	2.0	6.0	1.3	20.0
35. McGill Smith 5266	—	—	—	—	2.9	—
36. McKelvey 675	5.3	—	—	—	—	—
37. McKelvey B. 612	—	—	—	9.0	—	—
38. President	—	5	6.0	—	—	37.0
39. Roderick Dhu	5.1	9	7.5	—	—	7.0
40. Shamrock	5.0	—	—	9.0	2.2	—
41. Skerry	4.8	2	—	0.0	—	—
42. Up-to-Date	5.7	1	3.0	8.0	2.0	22.0
43. X 9	5.3	—	—	—	—	—

* Reported as resistant.

(c) Susceptibility in the field.

In the three seasons 1930-2, thirty-five varieties were tested for field susceptibility by planting in soil contaminated with *P. erythroseptica*. The plot of land in which the 1930 and 1931 tests were carried out had borne a crop fairly heavily and uniformly attacked with pink rot in 1929. The land in which the 1932 tests were carried out had not borne a potato crop for many years. Here artificial contamination of the soil was employed, soil from the test plots of the previous two seasons being applied at a rate of 10-15 lb./20 sq. yd. at the time of planting. The variety plots in each season consisted of single drills 30 yards long in 1930 and 1931 and 22 yards long in 1932. The tubers were planted 12-15 in. apart in the drills. Farmyard manure and artificial manures (at a rate of approximately 20 tons farmyard manure, 1 cwt. ammonium sulphate, 1 cwt. muriate of potash and 4 cwt. superphosphate per acre) were applied. The crop was sprayed twice each season with 2 per cent. Burgundy mixture. The varieties used and percentages of diseased tubers found at the time of raising the crop are shown in Table V, columns 4, 5, and 6.

In order to ascertain the progress of the disease during storage the crop raised from each variety in 1930 was stored in a clamp. On examining the tubers after four months it was found that in most varieties the percentages of tubers affected with pink rot had greatly increased. The results of this examination are given in Table V, column 7.

The general results obtained from these experiments indicate that all the varieties tested are more or less susceptible to pink rot. In no case was any variety found to be highly resistant.

PERENNATION OF THE PARASITE.

Pethybridge⁽¹⁵⁾ came to the conclusion that *P. erythroseptica* could exist indefinitely in the field. He records a case in which a piece of land, which had previously borne a crop affected with pink rot, was laid down to grass for a period of four years and then again planted with potatoes; the resultant crop was affected to the extent of over 5 per cent. A similar case involving an interval of five years was recorded in County Antrim during the present investigation: the seed used in this case was from an unaffected crop and, so far as could be ascertained, no contamination of the manure with diseased material had occurred. Two further cases, each involving an interval of four years, were noted on the same farm.

The actual mode by which *P. erythroseptica* perennates in the soil seems to involve both the production of resting spores and existence as a saprophyte. Pethybridge⁽¹¹⁾ found that oospores were produced in the bases of the dead stems of the host, and considered that the perennation of the fungus in the soil was mainly brought about in this manner. The production of oospores in the dead host plant has been confirmed in the present work. In one particular case, twenty-five plants which had borne tubers affected with pink rot were carefully examined for the presence of oospores. In eighteen plants oospores were found to occur more or less plentifully in the pith at the bases of the dead stems and in the stolons; in two plants a few oospores were found in the tissues of the roots. A few oospores were also found immediately below the skin of some tubers which had been inoculated with the fungus and allowed to decay in the soil; Pethybridge also observed that oospores may ultimately be produced in tubers. On the other hand de Bruyn⁽⁵⁾ has shown that the fungus is able to grow vigorously, produce oospores and subsist indefinitely in sterilised soil of various types. These findings have also been confirmed in the present investigation. From further work which has been carried out it would also appear that, under favourable conditions, the fungus will grow in unsterilised soil and that it is able to attack tubers stored in such soil.

TRANSMISSION OF THE DISEASE.

The following observations have been made with regard to the transmission of pink rot.

Where conditions are relatively dry, transmission of the disease from diseased to healthy tubers does not occur. Ten healthy tubers of each of the six varieties British Queen, Arran Victory, Golden Wonder, Up-to-Date, Arran Consul, and Incomer, were inoculated with *P. erythroseptica*. The tubers of each variety were then placed in a separate "sprouting" box (such as is generally used by potato growers), and each box was filled with healthy tubers of the corresponding variety so that the inoculated tubers were completely covered. The experiment was carried out in a cool dry store. On examination after several days it was found that most of the inoculated tubers had become affected with the disease and ultimately, after several months, these tubers had become shrivelled and dry. During the whole course of the experiment none of the uninoculated tubers contracted the disease.

It has already been shown that, *provided conditions are sufficiently*

moist, the disease may spread rapidly in an affected crop during storage. Although in general practice a few slightly affected tubers would normally escape detection and be selected as healthy for storage, the results of the clamping experiment mentioned on p. 389, and also general observations on the spread of the disease in clamps, indicate that infection of the majority of the diseased tubers occurs during storage. In a clamping experiment Pethybridge⁽¹¹⁾ found that the disease was not communicated from the diseased to the healthy tubers.

It is suggested that, under moist conditions, the occurrence and spread of the disease in an affected crop during storage may be accounted for by the presence of the parasite in soil adhering to the tubers, or in the soil used in clamp construction, rather than through actual transmission from diseased tubers to healthy tubers.

The transmission of pink rot by the use of "seed" from an affected crop involves two possibilities: (a) the carriage of the parasite in the soil adhering to the tubers and (b) the planting of diseased tubers. The latter possibility will be obviated if well-stored and sprouted "seed" is used, since tubers affected with the disease will have become mummified by planting time and obviously unfit for use as "seed." Apart from the actual carriage of the parasite by tubers from an affected crop, the extent to which the disease will appear in the resultant crop will primarily depend upon the conditions governing infection. Pethybridge⁽¹³⁾ found that, when tubers from an affected crop were planted in fresh land, the proportion of affected tubers in the resultant crop was 0.03 per cent. In 1929 a similar case was noted in County Antrim; land, which had not been cropped with potatoes for at least twenty-five years, was planted with tubers from a severely affected crop and no trace of pink rot was found in the resultant crop. These results, however, do not necessarily give a measure of the extent to which the parasite may have been disseminated by the "seed."

That the fungus may be transmitted to non-contaminated soil through the agency of contaminated soil was demonstrated by the varietal susceptibility field trials carried out in 1932 (*vide* p. 395). Twenty-three potato varieties were planted in a field which had not carried a crop of potatoes for many years. The soil was a heavy loam with a slow draining subsoil and somewhat subject to waterlogging. Artificial contamination of the plots with affected soil at planting time (*vide* Table V) resulted in the crops from twenty-two of the varieties becoming affected with pink rot to the extent of 0.4–7.0 per cent., while no trace of the disease was found in the control plots to which contaminated soil had

not been added. Cotton(4) concluded that the land in which the first reported case of pink rot occurred in England became contaminated through the use of "London manure," which included a large proportion of vegetable debris.

CONTROL OF THE DISEASE.

Well-drained land, good cultivation, and low rainfall provide unsuitable conditions for the growth and development of *P. erythroseptica* in the field, while "clamping" under well-aerated and dry conditions prevents its spread during storage. In any discussion relating to the control of pink rot, attention to measures designed to procure such conditions is of primary importance.

Varietal resistance of the potato, in so far as it has been considered, does not indicate the way to a solution of the problem. Certain varieties are apparently more susceptible than the majority, but no conclusive evidence has been obtained to show that any variety is particularly resistant.

The transmission of the disease to uncontaminated soil is most likely to occur through the medium of soil carrying the parasite or through the application of contaminated manure, the use of which should be avoided. The adherence of contaminated soil to "seed" tubers is a very likely method of such transmission. Although it is possible for the disease to be transmitted by the actual "seed" tubers, this is not likely to occur when the affected crop has been stored under dry and well-aerated conditions, preferably in sprouting boxes, since affected tubers will obviously have become unfit for seed purposes by planting time. Whether surface disinfection of seed tubers before planting, in order to kill the parasite present in the adhering soil, is likely to be successful and practicable has yet to be determined; experiments dealing with this aspect of control are in progress.

It has been demonstrated that oospores are freely formed in the tissues at the bases of the haulms of the ripening plants. The distribution of such infected haulms through the soil will obviously increase the prevalence of the fungus in the soil. The removal and destruction of the haulms immediately after ripening should, therefore, materially reduce this source of contamination. Incidentally this measure is also one of the recommendations made to secure the control of late blight (*Phytophthora infestans*).

Even though it has been shown that the fungus can exist indefinitely

in the soil, yet it is probable that its prevalence will diminish if the land be rested from potato cultivation; in view of this as long a period as possible should be allowed to lapse between potato crops.

Much may be done to prevent the parasite causing "misses" in the crop and the occurrence of the "pink rot wilt" phase of the disease by providing favourable conditions for the rapid establishment of the plants. It has been shown that the best method for ensuring such establishment lies in the planting of healthy, strongly sprouted tubers.

Pethybridge⁽¹⁰⁾ observed that normal infection of the tuber occurred via the "heel" end. During the present work this observation has been repeatedly confirmed in the field. This indicates that, in the field, the parasite enters the tubers through the mother plant. Because of this, and also because of the production of resting spores in the tissues of the dead mother plant, delayed digging should be avoided, and the crop should be raised as soon after maturity as possible.

Prevention of serious losses caused by the disease during storage of the crop may be secured by paying attention to the efficient aeration and the avoidance of humid conditions in the clamp or store. Before storing the crop it is important that it should be harvested under as dry conditions as possible.

DISCUSSION.

Reports of the occurrence of pink rot, caused by *P. erythroseptica*, in the British Isles are numerous, and the results of a survey carried out in Northern Ireland indicate the widespread nature of the causal fungus. Normally the attack is of insufficient severity to attract particular attention, with the result that reports of its occurrence are necessarily sporadic. Work, at present unpublished, has shown that the incidence of the disease is primarily controlled by the moisture factor. This is in accordance with the fact that, because of the varying climatic and soil conditions in different parts of the country, the disease tends to be of local importance; the relatively heavy and evenly distributed annual rainfall in the west of Britain is particularly favourable for the occurrence of the disease in Ireland.

Pink rot is normally caused by *P. erythroseptica*. Except in the case of *P. erythroseptica* var. *atropae* Alcock, the species has shown no appreciable variation in its morphological and physiological characters. Although it has been shown that, after artificial inoculation, several other

species of *Phytophthora* can cause pink rot of the potato tuber, only two cases have been noted in which the cause of the disease in the field was other than *P. erythroseptica*, viz. *P. drechsleri* Tucker in the U.S.A. and *P. megasperma* Drechsler in Northern Ireland. This, and the fact that the coloration symptoms produced in a diseased tuber are a function of the tuber alone, indicate the importance of identifying the parasite in cases of pink rot—especially in the case of a “first record” for any particular district or area.

Although pink rot is normally a disease of the tuber, the causal fungus may give rise to a systemic disease of the potato plant whereby it causes pink rot wilt. In such cases the plant probably becomes infected *via* the seed tuber, infection again depending upon the moisture content of the soil. Should the seed tuber be destroyed before the establishment of the plant then a “miss” occurs in the crop, but should the plant be weakly established, then the wilt aspect of the disease is produced. A healthy well-established plant appears to be highly resistant. As the plant matures its resistance decreases and during the ripening period it may become invaded by the fungus; growth of the fungus from the stolon into the tuber explains why the majority of the tubers become infected *via* the “heel” end. In cases where the disease occurs during storage it is interesting to note that infection generally commences at the “eyes” of the tubers.

Although, as de Bruyn⁽⁵⁾ has shown, *P. erythroseptica* is capable of saprophytic existence in the soil, yet it would appear that the persistence of the fungus in contaminated soil is principally due to resting spores. In artificial media the fungus is so readily overcome by other competing organisms that it is difficult to understand how it can have a vigorous saprophytic existence in a normal soil.

SUMMARY.

1. A survey of the occurrence and importance of pink rot of the potato is given. The disease is widespread in Britain, but is only occasionally of serious economic importance.

2. The nature of pink rot is explained and it is shown how the disease may be caused by more than one pathogenic fungus. *Phytophthora erythroseptica* Pethybr. is the normal pathogene, but two other species of *Phytophthora* have been recorded as causing the disease in the field, viz. *P. drechsleri* Tucker in the U.S.A. and *P. megasperma* Drechsler in Northern Ireland.

3. The disease may be systemic in the potato plant, causing "misses" and "pink rot wilt" in the crop; normally, however, it is of importance only as a disease of the tuber.

4. Under conditions of high humidity and poor aeration the disease may cause heavy loss in an affected crop during storage.

5. Under normal conditions in the field the tuber becomes infected *via* the mother stolon; if conditions in the field or store are sufficiently moist, however, the tuber becomes infected directly, generally through the "eyes."

6. Varietal susceptibility of the potato has been considered on the three bases: (a) artificial infection of the tuber with *P. erythroseptica*, (b) natural infection of the tuber when stored in contaminated soil, (c) susceptibility in the field when planted in contaminated soil. Altogether fifty-one varieties (including nine mentioned as susceptible by Pethybridge and which were not tested) have been considered, all of which proved to be more or less susceptible. No variety was found to be particularly resistant.

7. Large numbers of oospores are produced by *P. erythroseptica* in the haulms and stolons and also to some extent in the roots of the dead potato plant. These oospores become disseminated and greatly increase the prevalence of the fungus in the soil.

8. It has been shown that, in the absence of the host plant, the fungus may persist indefinitely in the soil. This persistence is apparently principally due to the resting spores, but the fact that the fungus may exist to some extent as a saprophyte in the soil cannot be ruled out.

9. The disease may be satisfactorily controlled in practice by paying attention to the general principles of crop hygiene. Satisfactory crops can be grown in contaminated soil, provided that strongly sprouted tubers are planted and that soil drainage is sufficiently good to prevent the land from becoming unduly wet in times of heavy rainfall. Early harvesting of the crop tends to secure a minimum percentage of affected tubers at the time of digging. Prevention of the spread of the disease during storage may be obtained by ensuring dry and well aerated conditions in the store.

The writers wish to express their very sincere thanks to Mr A. Murray, Culnafay, Toomebridge, County Antrim, who has given every facility for the carrying out of field experiments on his farm, and to the Potato Inspectors in Northern Ireland who assisted in recording occurrences of the disease.

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EXPLANATION OF PLATE XXII.

Illustrating representative plants selected from the series of tuber inoculations made with *P. erythrosetptica*.

- A, control—tuber wounded but not inoculated.
- B, tuber inoculated when the shoot was 2 in. above soil level.
- C, tuber inoculated when the shoot was 6 in. above soil level.
- D, tuber inoculated at the "heel" end at the time of planting.
- E, tuber inoculated at the "rose" end at the time of planting.

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AN EXPERIMENT ON THE INCIDENCE AND SPREAD OF ANGULAR LEAF-SPOT DISEASE OF COTTON IN UGANDA

By C. G. HANSFORD, M.A.

(*Department of Agriculture, Kampala, Uganda*),

H. R. HOSKING, B.Sc., A.R.C.S.

(*Department of Agriculture, Serere, Uganda*),

R. H. STOUGHTON, D.Sc.

(*Department of Mycology, Rothamsted Experimental Station, Harpenden*),

AND

F. YATES, B.A.

(*Department of Statistics, Rothamsted Experimental Station*).

(With 8 Text-figures.)

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I. DESIGN OF THE EXPERIMENT.

THE experiment to be described was designed to test the possibility of investigating the incidence and spread of the angular leaf-spot disease of cotton under field conditions, and of obtaining results which should be susceptible of statistical treatment. A great part of the knowledge of the dissemination of the disease and the influence of climatic conditions on this spread is purely empirical or deduced from laboratory studies, and this experiment, which is the first of a series, was undertaken to explore

the difficulties of putting this knowledge on a more exact foundation. It is intended to continue these experiments, modifying them from year to year as experience shows to be necessary, and thus to build up a collection of data from which definite conclusions may be drawn. The form and lay-out of the experiment was designed at a conference at Rothamsted in 1931, and duplicate experiments were carried out at Kampala and at Serere in Uganda during the cotton season 1931-2. Certain difficulties arose at Serere in the taking of the records, chiefly due to pressure of work, and in addition the spread of the disease at this station was so rapid as to obscure differences between plots very early in the season. For these reasons it has been thought best to confine attention in this paper mainly to the Kampala experiment, giving a summary only of the salient points from the Serere results.

The lay-out of the experiment at Kampala was in the form of a 4×4 Latin square, chosen by random selection (see Fig. 1). Each plot consisted of eleven rows of plants, each 3 ft. by 3 ft., giving a total area of just under half an acre. The experiment was on a moderate slope, the south corner being the highest. This corner was also the poorest with regard to depth of soil, and the soil in this part became very hard in dry periods.

Four seed treatments were used:

A. Untreated seed.

B. Seed dusted with "Granosan" (supplied by the Dupont Corporation, U.S.A.).

C. Seed delinted for 40 min. with concentrated sulphuric acid, washed, and then sterilised by immersion in 1 per cent. mercuric chloride solution under a partial vacuum for 15 min. and washed again. By this treatment it was hoped completely to avoid infection from *B. malvacearum* carried on the seed coat.

D. Seed soaked in a strong suspension of *B. malvacearum*.

1 B	2 D	3 C	4 A
5 C	6 A	7 D	8 B
9 D	10 B	11 A	12 C
13 A	14 C	15 B	16 D

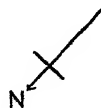


Fig. 1. Lay-out of experiment. Treatments: A. Control, untreated. B. Dusted with "Granosan." C. Delinted and sterilised. D. Inoculated.

The whole area was sown on August 5th after a few days' rain, but unfortunately no further rain fell for a fortnight, so that germination was slow and irregular. After germination the seedlings were thinned to two per hole, until the unfolding of the first two true leaves, when the plants were singled. Owing to the delayed germination this final thinning was not completed until towards the end of September. The first flowers appeared on the plot about October 20th, and the first picking took place on December 27th, the final picking being made on February 8th.

Observations on disease were made every 2 days up to September 21st, then twice weekly, and towards the end of the season at longer intervals. Each individual plant was examined for cotyledon, leaf, or stem infection and a plan made for each observation of the experiment to show the situation of the infection in the plots. Up to the time of singling the examination was made on a hole basis, that is, a single seedling in one hole showing infection was recorded as an infected hole. The three types of attack, cotyledon, leaf-spot, and stem lesion, were recorded separately as primary, secondary and black-arm infections.

Throughout the experiment careful meteorological records on the site were kept. These included observations of dry- and wet-bulb temperatures at 8.0 a.m. and at 2.0 p.m. local time, maximum and minimum temperatures, rainfall, and evaporation as measured by a Piché evaporimeter. In addition the full official records from Kololo Hill Observatory near Kampala were available.

The crop from each plot was collected in separate bags and weighed after the final picking. The number of bolls picked from each plant was recorded on a plan similar to those used for the infection records, no account being taken of boll diseases and pests. In future work it is hoped to elaborate the crop records in respect of such matters as the ratio of healthy to diseased bolls, clean to stained cotton, and so on. In the present case, however, it was deemed advisable to simplify crop records as much as possible, and to eliminate factors other than the disease under investigation. At Serere the lay-out of the experiment was similar to that at Kampala, but only the final yield figures and the number of plants are considered in this paper.

II. COURSE OF THE DISEASE.

(1) *Primary infection.* Fig. 2 shows the course of development of the primary infection at Kampala. Figs. 2, 3, and 4 have been prepared from the original dot-plans showing the location of the infected plants at each observation, and represent the distribution of the disease at

weekly intervals for the first half of the season, and at longer intervals later. As was to be expected, the primary infection was largely confined to the inoculated plots, although a few cases occurred on the control (untreated) areas. The seed was derived from a not very heavily attacked crop and so would not be expected to carry much natural infection. This

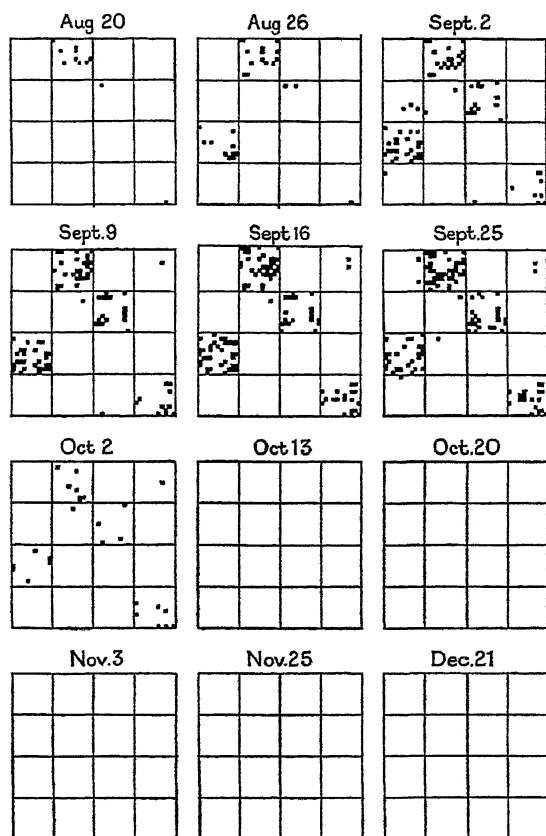


Fig. 2. Distribution of primary infection on different dates.

phase of the disease reached its maximum about the third week in September, 26 per cent. of the plants on the four inoculated plots showing infection on September 25th. The cotyledons were shed at this time, and the recorded primary infection decreased rapidly, becoming zero on October 13th when all cotyledons had fallen. The course of the primary infection on the inoculated plots is shown graphically in curve V of Fig. 6, as the mean percentage infection for the four plots of the treat-

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ment at each date. Fig. 2 shows that no spread from plot to plot occurred during this phase of the disease. In view of the small size of the plants at this stage and their relatively large distance from one another,* it would not be expected that such spread would occur.

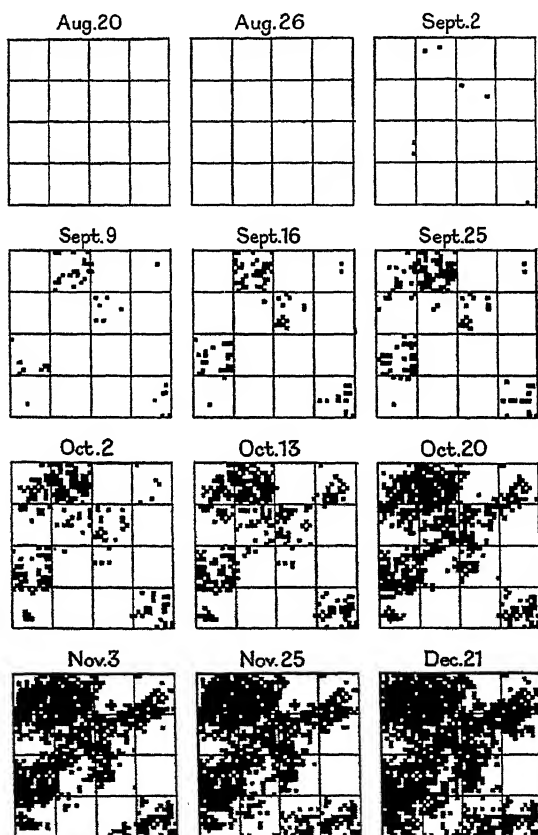


Fig. 3. Distribution of secondary (leaf-spot) infection.

(2) *Secondary infection.* The distribution and spread of the secondary infection is shown in Fig. 3, while the development is shown graphically in Fig. 6. Up to September 16th the secondary attack followed almost exactly the course of the primary infection. On that date there were only five plants outside the inoculated plots showing any infection, although on the latter 20 per cent. of the plants were attacked. Nine days later, however, on September 25th, infection had begun on plots 1 and 5, and by October 2nd these plots and also plots 6 and 13 were fairly

heavily attacked. Examination of the plan in Fig. 3 for these dates leaves little room for doubt that this infection spread in from the adjacent inoculated plots. The spread from the infected plots did not, however, occur in all directions equally. Clear examples of this are

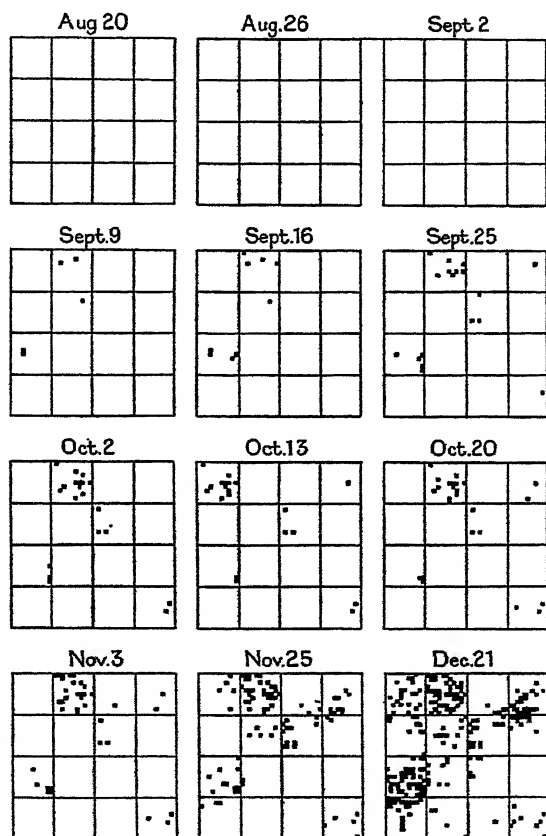


Fig. 4. Distribution of plants with black-arm infection.

given by plots 3 and 12. Plot 3 is bounded on the north-east by the very heavily infected plot 2, yet little spread occurred in this direction although plot 1 on the other side became severely attacked. Plot 12, again, is bounded to the north-west by plot 16, another inoculated area, but remained clean throughout the season. In general, there was a clear tendency for the spread to occur in a northerly direction, that is, down the slope of the land. This directional spread might be explained by one or more causes. An analysis of the prevailing winds, as far as the data

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available allows, gives little indication of any decided preference for one direction, and we cannot definitely attribute the dissemination to this factor. Two other factors, both due to the slope of the ground, may be involved. In the first place, during a heavy rainstorm the splash droplets, which have been shown by Faulwetter(1) to consist of the water of the surface film on the leaf and thus to be the carrying agents of the disease, will tend on the whole to convey the infection down the slope rather than

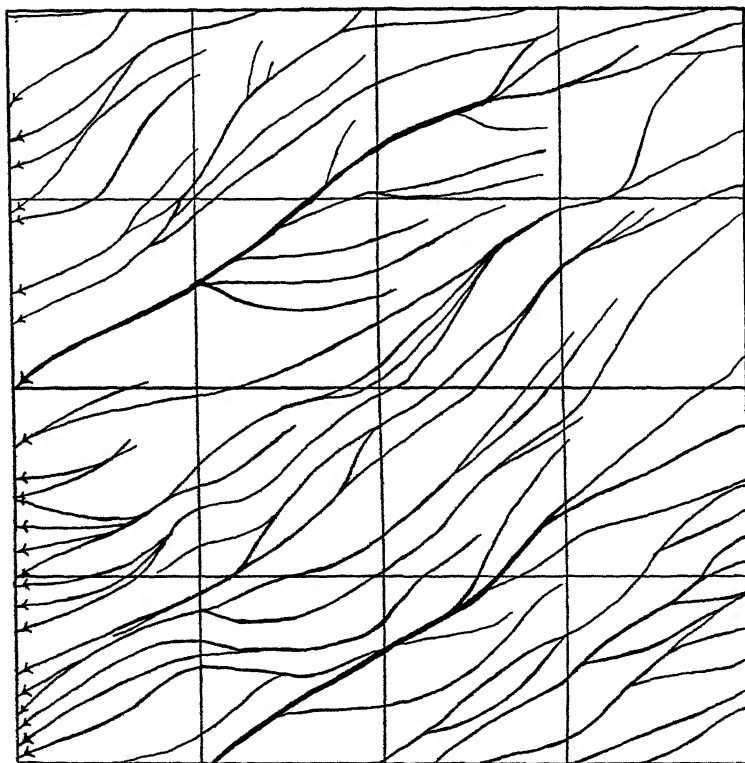


Fig. 5. Chart of main channels of surface-wash, October 5th.

up, in the absence of any strong counter-wind. In the second place, the infection might be carried in the water running over the surface of the ground and splashed up by falling rain. A chart of the main lines of surface wash is given in Fig. 5. This was made immediately after a very heavy fall of rain, and it will be seen that the direction of the lines follows very generally the direction of spread of the disease. This point is of interest in connection with the later discussion on the influence of meteorological conditions on the disease.

In spite, however, of the extensive spread of the disease, there remained a very marked difference in the degree of infection on the different plots. Curves I, II, III and IV of Fig. 6 show the course of the mean percentage infection for the four plots of each treatment. The eight

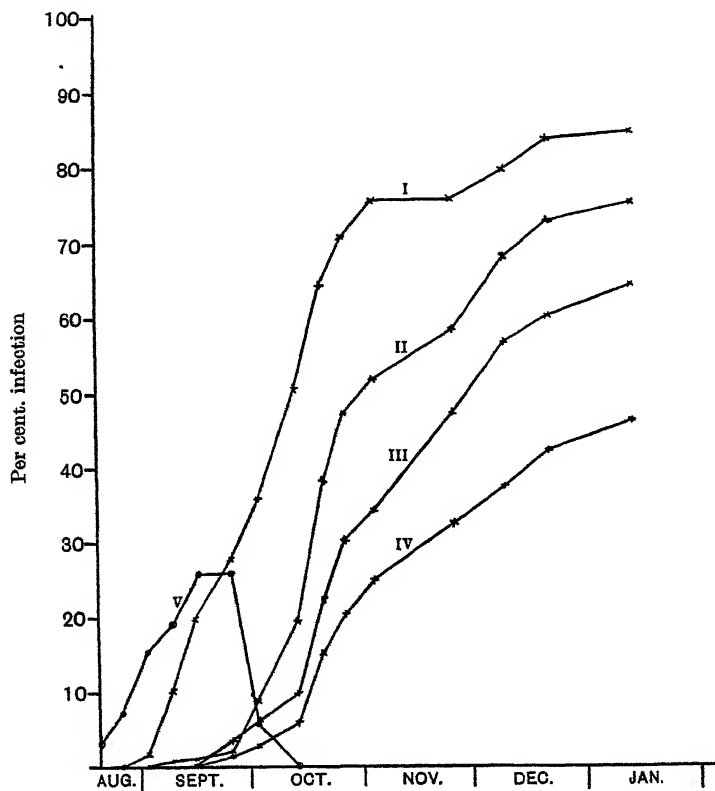


Fig. 6. Development of primary and secondary (leaf-spot) infection. Curve I, mean secondary infection treatment D (4 plots). Curve II, mean secondary infection treatment A (4 plots). Curve III, mean secondary infection treatment B (4 plots). Curve IV, mean secondary infection treatment C (4 plots). Curve V, mean primary infection treatment D (4 plots).

plots sown with treated seed show a marked reduction in the amount of disease, the delinted seed giving the healthiest crop. This difference in favour of the delinted seed is partly due to the complete freedom from attack of plot 12, a freedom which, as pointed out above, seems to be due largely to its position. The influence of the disease on the final yield will be discussed later in this paper.

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(3) *Black-arm or stem infection.* Fig. 4 shows the distribution of plants having black-arm lesions, on the same dates as for the other phases of the disease, while the development of this phase is shown graphically in Fig. 7. The first point of note is that black-arm lesions were found only on plants previously recorded as showing angular leaf-spot. This manifestation of the disease occurs much later in the season than the leaf-spot type under Uganda conditions, although a few plants on the inoculated plots showed lesions quite early. It follows from the point noted that no independent spread of this phase of the disease was found, the attack being evenly distributed among the plants with leaf-spot. Arguing further from this it is clear that the amount of black-arm

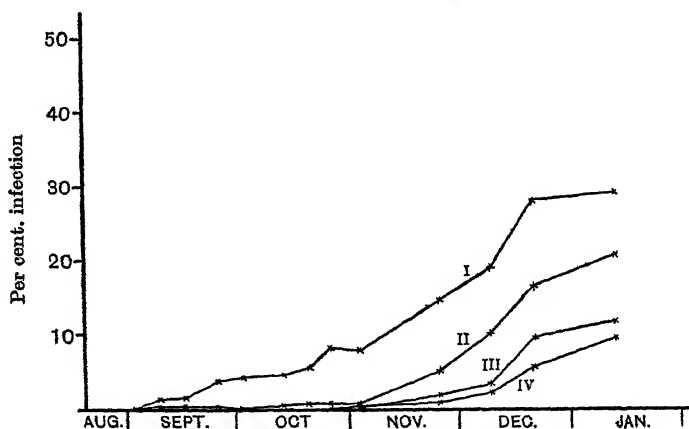


Fig. 7. Development of black-arm infection. Curve I, mean infection treatment D (4 plots). Curve II, mean infection treatment A (4 plots). Curve III, mean infection treatment B (4 plots). Curve IV, mean infection treatment C (4 plots).

attack on the plots of the different treatments would be expected to be in the same order as for the secondary attack; Fig. 7 shows that this was the case, the inoculated plots having the greatest infection and those sown with delinted seed the least. In view of the previous statements made by one of us (3) as to the origin of stem infections, an analysis of all infections on the stem and branches of the plants was made at the last four observations. A summary of the results is given in Table I. It will be seen that these figures bear out the theory that the majority of the lesions on stems and branches are extensions of lesions on the petioles. These petiolar infections, in turn, originate in the leaf, the organism spreading downwards through the cortical tissue of the leaf-stalk and reaching the cortex of the stem or branch. A point of interest

to note in the figures is the relatively small increase in the number of lesions on main stems in comparison with the increase in the number on branches and short shoots arising from secondary buds. This is in conformity with the rule that the organism can obtain a foothold only in young, immature tissues.

Table I.

Site and origin of black-arm lesions at four final examinations.

Treatments	Site					Origin			
	Stem	Branches	Short shoots	Boll wall	Boll pedicel	Petiole	Stipules	Bracts	Others
November 25th									
Untreated A	7	18	0	0	1	24	0	1	0
Dusted B	5	7	0	0	0	11	1	0	0
Delinted C	2	2	0	0	0	4	0	0	0
Inoculated D	49	39	1	0	0	84	1	0	3
December 9th									
Untreated A	10	52	14	0	3	74	2	3	0
Dusted B	5	18	4	1	3	26	1	3	0
Delinted C	2	8	0	3	1	8	1	1	0
Inoculated D	56	68	16	2	1	137	0	1	3
December 21st									
Untreated A	14	113	49	36	(6)	167	0	6	3
Dusted B	5	51	16	13	(5)	68	1	5	0
Delinted C	2	31	7	10	(1)	39	0	0	1
Inoculated D	58	139	61	39	4	249	3	4	6
January 16th									
Untreated A	14	167	76	31	(6)	248	0	6	3
Dusted B	6	79	48	12	(4)	128	1	5	0
Delinted C	2	44	17	15	1	63	0	0	1
Inoculated D	58	173	79	23	4	302	2	4	6

III. INFLUENCE OF METEOROLOGICAL CONDITIONS.

Fig. 8 shows the variations in the main meteorological conditions during the season, together with a curve showing the mean percentage secondary infection for the whole area. It is at once apparent that little or no definite information can be gleaned on the possible correlation between increase in disease and the main climatic factors. The variations in maximum and minimum temperatures were too small and too close together in point of time to be reflected in the disease curve, while the rainfall was too evenly spread over the growing season for any clearly marked correlation with the curve for disease to be evident. The latter shows two fairly well-pronounced rapid rises, the first between October 2nd and October 20th, and the second between November 25th and December 9th. While it is true that both of these rises follow periods of heavy rainfall about a week earlier, on the other hand the 3 days' heavy

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rain of October 22nd-24th is not reflected in a subsequent increase of infection.

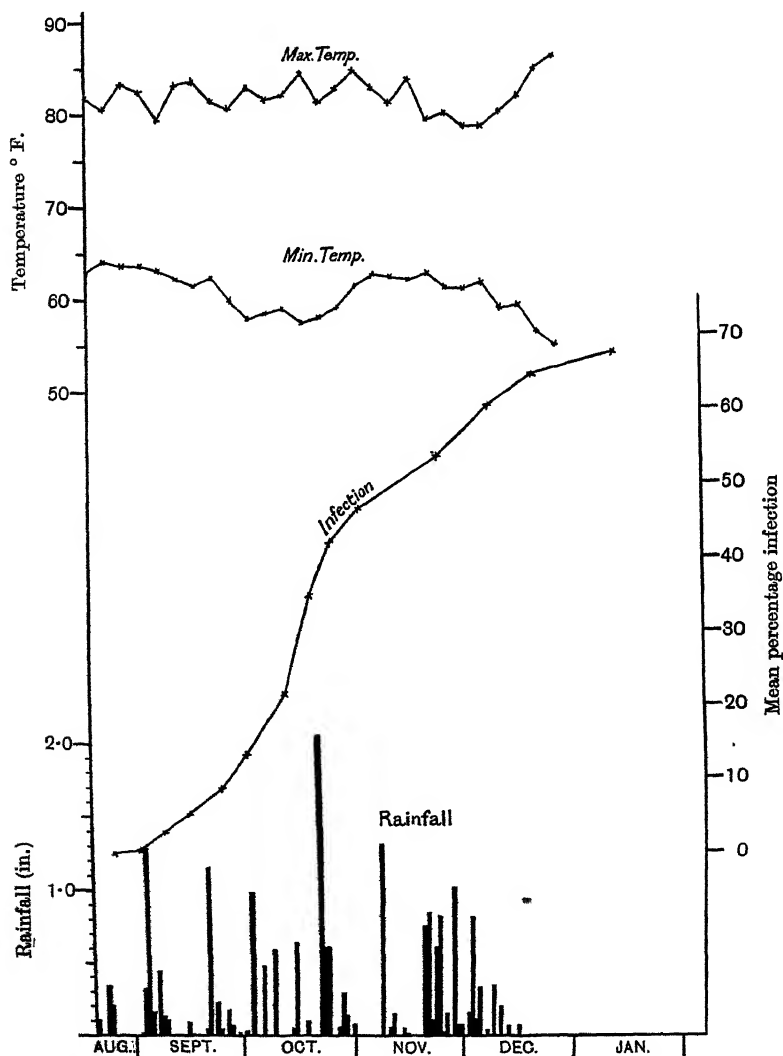


Fig. 8. Chart of rainfall (actual falls), maximum and minimum temperatures (smoothed 5-day means plotted each 5th day) and percentage secondary infection (mean of all plots).

It is clear, in any case, that a single experiment could not give reliable information on such a complex problem as the influence of climatic

conditions on disease. It is only by the accumulation of results over a number of years that an analysis of the separate factors becomes possible. It is hoped that the series of experiments, of which this is the first, will provide the necessary material for such an analysis. The general implications of the experiment and the suggestions that it gives for future modifications will be considered in the discussion at the end of this paper.

IV. STATISTICAL ANALYSIS OF RESULTS.

The two experiments, at Kampala and Serere, may best be considered together from the point of view of statistical analysis. The data provided by the Serere experiment is meagre, only the plant number, the weight of seed cotton, and the weight of the diseased bolls (which gave no cotton), being available for the individual plots. In the Kampala experiment the total yield of seed cotton, the plant number, and the number of "infection days" for angular leaf-spot and black-arm, are all available for the individual plots. These are given in Table II, and the corresponding figures for Serere in Table III. The number of infection days for each phase of the disease is obtained by computing the total number of days of infection of all infected plants, that is, the number of new infections at each observation multiplied by the number of days remaining to the end of the experiment. It should be emphasised, however, that the degree of replication (a single 4×4 Latin square at each centre)

Table II.
Yields and disease data of individual plots.

Kampala.

Plot	No of plants	No. of bolls	Total yield oz.	Infection days	
				Angular leaf-spot	Black-arm
1	113	378	48	8627	739
2	108	306	34	10951	3271
3	88	161	18	2123	215
4	83	167	17	3718	941
5	82	214	26	5610	332
6	99	224	25	7331	412
7	99	195	20	6751	995
8	108	200	23	2090	342
9	108	291	29	9512	2155
10	116	245	29	4840	263
11	103	238	28	3643	138
12	106	294	36	0	0
13	105	299	34	5214	740
14	101	286	32	1754	93
15	113	214	25	2641	23
16	99	152	19	5551	530

Table III.
Yields of individual plots.
*Serere.*¹

Plot	No. of plants	Yield of seed cotton oz.	Weight of diseased bolls lb.	Plot	No. of plants	Yield of seed cotton oz.	Weight of diseased bolls lb.
1	103	9.3	1.50	9	99	13.6	2.50
2	109	8.5	1.66	10	110	13.1	3.13
3	104	13.6	2.84	11	106	17.6	3.81
4	114	23.0	4.16	12	113	14.6	5.63
5	106	10.1	2.50	13	114	12.5	2.00
6	106	12.0	2.50	14	99	8.0	1.66
7	117	15.5	3.19	15	114	10.9	3.00
8	111	18.4	4.50	16	115	17.8	5.53

is scarcely sufficient to provide any very precise amount of information on such questions as the connection between yield and disease.

Table IV.
Totals of yield and plant number for the different treatments.
Kampala.

	Un-treated	Dusted	De-linted	Inocu-lated	Mean	Standard error
Plant No.	390	450	377	414	407.8	14.9
Yield (oz.)	104	125	112	102	110.8	16.7
Correction for plant No.	+16.7	-39.7	+28.9	-5.9	—	—
Corrected yield	120.7	85.3	140.9	96.1	110.8	9.93

Increase in yield on plots treated alike for each additional plant (b) = +0.9402.

Serere.

	Un-treated	Dusted	De-linted	Inocu-lated	Mean	Standard error
Plant No.	424	455	442	419	435	7.16
Yield (oz.)	57.0	64.2	44.0	53.3	54.6	4.29

The totals of yield and plant number in the different treatments are given in Table IV. At neither centre does an analysis of variance(2) of the yields, unadjusted for plant number, give any significant results, the standard errors per plot having the high values of 15.1 and 15.7 per cent. respectively. In the analysis of plant number, however, given in Table V, the effect of treatments almost reaches the 5 per cent. level of significance at Kampala and passes it at Serere. This is due at both stations to the high number of plants on the plots with dusted seed.

¹ As the Serere results are not considered in detail, the key-plan of treatments at this station has not been included. The lay-out was similar to, but not identical with, that at Kampala.

Taken together the two experiments show a significant increase in plant number with the dusting of the seed, there being no significant differences between the other treatments. This result confirms claims previously advanced that dusting improves total germination.

Table V.
Analysis of variance of plant number.
Kampala.

	Degrees of freedom	Sum of squares	Mean squares	<i>z</i>
Rows	3	345.19	115.06	—
Columns	3	106.19	35.40	—
Treatments: Dusted v. others	1	595.03	595.03	1.184
Others	2	176.17	88.08	0.229
Total	3	771.20	257.06	0.763
Error	6	334.37	55.73	—
Total	15	1556.94	—	—

Serere.

	Degrees of freedom	Sum of squares	Mean squares	<i>z</i>
Rows	3	37.00	12.33	—
Columns	3	162.50	54.17	—
Treatments: Dusted v. others	1	133.33	133.33	1.170
Others	2	73.17	36.56	0.524
Total	3	206.50	58.83	0.840
Error	6	77.00	12.83	—
Total	15	483.00	—	—

z (5 % point, 3 D.F., 0.780; 1 D.F., 0.895.
1 % point, 3 D.F., 1.140; 1 D.F., 1.310.

A further analysis of yield was made at both stations by eliminating the effect of plant number by an analysis of covariance(2). The Kampala analysis is given in Table VI. At Serere there was no appreciable regression of yield on plant number, and the analysis is without interest. At Kampala, however, the regression of yield on plant number is undoubtedly significant, the regression coefficient having the very high value of + 0.9402, indicating an increase of yield of this amount for each additional plant above the mean number of plants, although the mean yield per plant over the whole experiment is only 0.271. This can only mean that plant number is in some way correlated with the fertility of the plots, so that plots with a higher plant number yield more *per plant* than those with a lower plant number receiving the same treatment. This is not in fact unreasonable, since the chief cause of loss of stand was due to drought when the plants were young, and plots non-resistant to

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this drought might be expected to be less resistant to later droughts, and also to contain a greater proportion of damaged plants.

Table VI.

Analysis of covariance of yield and plant number at Kampala.

	Degrees of freedom	Sums of squares and products				Mean square
		Plant No. (N)	$N \times Y$	Yield (Y)	$Y \cdot bN$	
Rows	3	345.19	127.19	111.69	177.67	59.22
Columns	3	106.19	108.94	353.19	242.21	80.74
Treatments	3	771.19	157.19	81.69	467.84	155.95
Error	5	334.37	314.37	418.87	123.31	24.66
Total	14	1556.94	707.69	965.44	1011.03	

Since dusting has an effect on plant number the reduction of the yields of the different treatments to the basis of constant plant number, by means of the regression coefficient 0.9402, has no real validity. To make such a reduction valid it would have to be assumed that the high plant number of the dusted treatment was merely an indication that this treatment had fallen on a particularly favourable set of plots. Such an assumption is clearly unjustified. The reduction is shown in Table IV merely as an illustration of statistical procedure, and to emphasise the danger of an uncritical application of the methods of covariance to the elimination of plant number when the treatments themselves affect plant number. It will be noted that the yield from dusted seed, which was the highest, is now the lowest. The differences in corrected yield would be judged significant by the application of a z test to the mean square values of Table VI.

In order to see if there was any connection between yield and disease on similarly treated plots the partial regressions of the yield on the number of angular leaf-spot "infection days" and on the plant number were computed by the method of the analysis of covariance. The residual covariance between infection days and plant number happens to be zero (showing no connection between disease and plant number on plots treated alike), and the two partial regressions are therefore independent. The regression coefficient of yield on infection days is + 0.2591, showing an apparent positive correlation between yield and disease, but this correlation is by no means significant, since the amount of the sum of squares accounted for is only 31.45. There is thus no indication of any connection between yield and disease, as measured by the number of angular leaf-spot infection days. Similar results are obtained by taking the number of black-arm infection days as a measure of disease.

The analysis of the number of angular leaf-spot infection days shows

that the inoculated plants have a significantly higher, and the delinted plants a significantly lower, incidence of disease than the other two treatments. The lesser infection of the delinted as compared with the dusted plots may be in part a positional effect. These differences persist in the black-arm phase of the disease. The results are shown in Table VII.

Table VII.
Number of infection days.

	Untreated	Dusted	Delinted	Inoculated	S.E.
Angular leaf-spot	19906	18198	9487	32765	± 1770
Black-arm	2231	1367	640	6951	—

V. DISCUSSION.

It was pointed out in the introductory part of this paper that the experiment was of a preliminary nature, and was designed to investigate the possibilities of carrying out a field test of the spread of a disease and of its effect on the crop, which should be susceptible of statistical analysis. While it was not to be expected that this first experiment would lead to any definite conclusions, yet certain indications emerge of lines worth further study, and the analysis of the results offers suggestions for future modifications in the technique.

It is clear that the seed treatments employed have had a marked effect both on the germination of the seed as shown by the final stand obtained, and on the incidence of the disease. Treatment of the seed with a bactericidal dust resulted in a significant increase in the total germination, while sterilisation with sulphuric acid and mercuric chloride greatly reduced the amount of the disease in all its phases. It is perhaps unfortunate, from the point of view of seed treatment, that so little primary infection occurred on the untreated seed. On the other hand, the fact that this primary attack was practically limited to the inoculated plots gave better opportunity for observing directional spread. There can be no doubt that the spread of the disease has some relation to the lie of the land and the consequent surface wash. It was hoped at the inception of the work to obtain some information on the correlation between the incidence and spread of the disease and meteorological conditions, especially rainfall, but the distribution of the rainfall during the season was too uniform to give more than slight indications of its effect on the attack. In any case, experiments repeated over several years will be necessary before any exact analysis of the variable climatic factors can be made.

A further point on which it is hoped these experiments will give information is the effect of the disease on the final yield. Here the great

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difficulty is to obtain a single figure for a plot which will give a measure of the degree of incidence of the disease. The final percentage infection at the time of picking is hardly a fair measure, since some plants may have been attacked very late in the season when the disease will have little effect on the final yield. The figure adopted of "infection days" would appear to give the fairest estimate, since, *caeteris paribus*, early infection persisting throughout the season is more likely to influence the yield than a late infection. The adoption of "infection days" as the measure gives due weight to the time factor in disease. The present experiment does not give any indication of a correlation between yield and disease. In future work it is proposed to separate the two investigations of the effect of disease on yield and of the influence of climatic factors on the disease, and two entirely different experiments to study these problems have been devised and are now being carried out in Uganda. It is hoped by divorcing the two distinct problems to obtain more precise information on each.

VI. SUMMARY.

Experiments on the incidence and spread of the angular leaf-spot disease of cotton, carried out at two centres in Uganda, are described.

Treatment of the seed by sterilisation with sulphuric acid and mercuric chloride resulted in a reduction in the amount of the disease throughout the season.

Treatment of the seed with a bactericidal dust had a significant effect on total germination, the plots sown with this seed having a greater number of plants at the end of the season, independently of those killed by the disease.

Primary infection was almost entirely limited to plots sown with seed inoculated with the organism.

Spread of the disease occurred in a direction down the slope of the ground and along the lines of surface wash.

The implications of the experiment are discussed and proposals made for modifications in technique.

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AN INVESTIGATION OF TOMATO VIRUS DISEASES OF THE MOSAIC "STRIPE," STREAK GROUP

By G. C. AINSWORTH, B.Sc.

(*Experimental and Research Station, Cheshunt, Herts.*)

(With Plates XXIII and XXIV.)

INTRODUCTION.

TOMATO virus diseases of the "stripe" or "streak" type have long been known, but there is still confusion as to their identity and relation to "mosaic." This group of diseases as occurring in glasshouses in this country has been examined. Several distinct viruses were found to be involved and they are briefly described and compared in this paper.

ORDINARY OR MILD TOMATO MOSAIC.

This is the most commonly occurring mosaic of tomato in the British Isles.

Material obtained from the Experimental Station nursery and several commercial nurseries was compared with authentic tobacco mosaic (tobacco mosaic No. 1 of Johnson(8)), kindly supplied by Dr Henderson Smith of Rothamsted, with which it was found to be identical. This agrees with the work of Allard(2) and others in America.

Table I summarises the filterability, resistance to ageing, heat and chemicals of these two viruses as determined by a series of parallel tests.

Table I.

Filterability and resistance to ageing, heat and chemicals of ordinary or mild tomato mosaic (tomato mosaic) and tobacco mosaic.

Filterability	Can be filtered unchanged through L_3 , L_5 , and L_7 Pasteur-Chamberland filters
Resistance to ageing <i>in vitro</i>	Several years*
T.D.P.	10 min. at 90° C.
Resistance to chemicals	Withstand 90 % alcohol or 1 in 200 nitric acid 24 hours

* For tobacco mosaic from Johnson's paper (8).

They have the same host range and produced identical symptoms on all the host plants used. The host plants employed were: *Lycopersicum esculentum*, *Nicotiana tabacum*, *N. macrophylla*, *Datura Stramonium*, *Solanum melongena*, *S. ciliatum*, *Physalis pubescens*, *Capsicum annuum*, *Petunia* sp., and *Cucumis sativa*. *Cucumis sativa* could not be infected but on all the other plants, except *Datura Stramonium* and *Nicotiana glutinosa*, the symptoms were a mottle of varying intensity together with some malformation and stunting. For purposes of comparison the symptoms on a few hosts will be briefly described.

Lycopersicum esculentum, tomato. When inoculated by friction with cheese cloth moistened with infected juice the percentage of infection is high (see Table III) and the incubation period varies from 5 days in summer to 3 weeks in winter. Under spring and summer conditions a mild to moderately severe dark green mottle with slight leaf distortion and stunting results. (If the plants are growing well the symptoms may become marked after the primary phase until the plants are "stopped" when developing side shoots are mottled and deformed.) There is no necrosis and the fruit is not marked. In winter the mottle is absent but stunting and leaf distortion are more severe, giving the "fern leaf" type of symptom, and there is a development of anthocyanin in the stem.

Nicotiana tabacum, tobacco. No necrotic local lesions are produced and the infection is invariably systemic. A slight clearing of the veins is followed by a dark green mottle and a degree of stunting and leaf distortion dependent on the season (see Fig. 2).

Nicotiana glutinosa. Necrotic local lesions develop but infection never becomes systemic and the virus cannot be re-isolated.

Datura Stramonium. Numerous necrotic local lesions are produced but infection is never systemic. In some cases dark brown lesions develop on the petiole, and stem below the node, of an inoculated leaf.

"STRIPE."

The symptoms on tomato of the disease generally known in this country as "stripe" are well known. "Stripe is characterised by brown longitudinal markings or stripes on the stem, by shrivelling of the leaves and by sunken irregularly shaped pits usually of a brown colour on the fruit" (Paine and Bewley⁽⁹⁾, p. 183).

This symptom picture may however be due to several causes—a bacterium (Paine and Bewley⁽⁹⁾), a single virus (Jarrett⁽⁷⁾ and this paper), or a mixed virus (Valleau and Johnson⁽¹²⁾, Burnett and Jones⁽⁴⁾, Jarrett⁽⁷⁾ and this paper).

NOMENCLATURE.

As it is usually impossible to distinguish these diseases on tomato they will doubtlessly as a group in this country retain the name "stripe." In this paper the diseases will be distinguished as follows. That attributed to *Bacillus lathyri* will be called *stripe* (Paine and Bewley(9)), that due to the single virus described below will be called *glasshouse streak* (Jarrett(7)), while *streak* (= experimental streak of Jarrett(7)) in conformity with American terminology will be reserved for a mixed virus infection.

GLASSHOUSE STREAK.

"Striped" tomato plants were obtained from over as wide an area as possible and inoculations made to tomato and tobacco plants. The same virus was found to be present in every case either alone or mixed with tomato mosaic or more rarely spotted wilt. A selection of 10 strains of this virus (from different parts of England, Ireland and Wales) was made and they were compared with each other and with tomato mosaic.

As regards filterability, ageing *in vitro*, and resistance to heat and chemicals the virus was found to be identical with tomato mosaic (see Table I). This is in agreement with the work of Jarrett(7). Though glasshouse streak was found to have a similar host range to tomato mosaic and on certain hosts produced identical symptoms, on other host plants the two viruses were readily differentiated. Jarrett(7) found a similar host range but with identical symptoms throughout. The symptoms on certain hosts will be described. *Nicotiana glutinosa*, *Datura Stramonium*, *Solanum ciliatum*, *Physalis pubescens*, and *Capsicum annuum* symptoms identical with tomato mosaic.

Lycopersicum esculentum, tomato. The symptoms agree with those given for stripe above (see Plate XXIII, fig. 1), but in addition there may be a mottle, identical with tomato mosaic mottle, which develops either before, at the same time, or after the "stripe" symptoms and which frequently has been the only symptom under the prevailing experimental conditions. The symptoms agree with one form of "tomato mosaic" described by Gardner and Kendrick(6).

Nicotiana tabacum, tobacco. Two to four days after inoculation necrotic lesions begin to develop which increase in size up to 5 or 6 mm. in diameter, while adjacent lesions may fuse to form large necrotic areas. This may be the only symptom (Plate XXIII, fig. 3 b). In some cases systemic infection follows at once (primary systemic infection) when a necrosis spreads rapidly down the petioles of the inoculated

leaves into the younger leaves (Plate XXIII, fig. 3 *a*). The plant is often killed but if it survives growth is very slow, the leaves are mottled and show necrotic lesions in the interneural areas (Plate XXIII, fig. 4). The plants which show only local lesions grow normally (Plate XXIII, fig. 3 *b*) and no virus can be isolated from the upper parts. Later, usually 2 or 3 weeks after inoculation, a number of these plants begin to show an internal necrosis of the stem which spreads rapidly upwards causing a vein necrosis of the apical leaves (secondary systemic necrosis, Plate XXIII, fig. 5). The virus can then be isolated unchanged. Growth of the plant is arrested and if growth is resumed it is generally due to the development of an axillary bud towards the base of the plant. Tables II and III summarise several series of tobacco and tomato inoculations.

Nicotiana macrophylla. Symptoms as *N. tabacum*.

Table II.

Summary of several parallel series of Nicotiana tabacum inoculations.

Virus	No. plants inoculated	No. showing local lesions	No. showing mottle only	No. showing systemic necrosis	
				Primary	Secondary
Tomato mosaic	12	0	12	0	0
	10	0	10	0	0
	12	0	12	0	0
Tobacco mosaic	12	0	12	0	0
	10	0	10	0	0
Glasshouse streak	24	24	0	18	—
	12	12	0	5	4

Table III.

Summary of tomato (var. E.S. 1) inoculations made over the same period.

Virus	No. plants inoculated	No. infected	Mosaic only	Leaf and stem lesions
Tomato mosaic	71	62	62	0
Glasshouse streak	79	79	57	22
Streak	58	58	0	58

Solanum melongena is another plant which sharply differentiates tomato mosaic and glasshouse streak. Tomato mosaic gives no local lesions and the systemic symptoms are a slight mottle and stunting (Plate XXIV, fig. 8). Glasshouse streak causes chlorotic areas or well-defined necrotic lesions on the inoculated leaves, and the systemic symptoms are a very slight mottle together with a severe necrosis of the veins causing the leaves to dry out and fall off. There is also some necrosis of the stem and petioles (Plate XXIV, fig. 7).

It can be seen from the above descriptions that in some respects the viruses of tomato mosaic and glasshouse streak are closely related. It is possible that the latter is only a more virulent strain of the former or that glasshouse streak is a mixed virus, but no observations in support of either view have been made. One strain of tomato mosaic has been under constant observation for eighteen months and several other strains for shorter periods, and in no instance has a variant arisen, the symptom picture remaining remarkably constant. Neither has a strain of glasshouse streak reverted to tomato mosaic. Tomato mosaic has not produced a case of "stripe" in tomato while glasshouse streak inoculations have given a mixture of mottled and "striped" plants (see Table III). This suggests that glasshouse streak is a mixture, but further inoculations have given both mottled and "striped" plants irrespective of the type of plant from which the inoculum was taken, and the virus has retained its identity on different hosts and under different treatments. Tomato mosaic and glasshouse streak retain their special properties when mixed. If tobacco plants are inoculated with a mixture of these two viruses the tomato mosaic can be recovered in a pure state, for though local lesions typical of glasshouse streak are produced, in those plants in which systemic infection of the glasshouse streak component does not take place or is delayed, typical tomato mosaic is exhibited in the younger parts. In a commercial crop there is frequently a mixed infection when the first inoculations from naturally infected plants to tobacco plants show local lesions followed by typical tomato mosaic symptoms, while subsequent inoculations from the young mottled tobacco leaves to tobacco show no lesions. If a series is continued in tomato from such a naturally infected plant then sub-inoculations to tobacco continue to show local lesions. The tobacco is able to filter out the glasshouse streak component.

STREAK.

The symptoms of streak are identical with those of glasshouse streak, but instead of a single virus a mixed virus is involved. Streak is typically the result of mixing tobacco mosaic and potato mosaic (see Dickson (5), Vanterpool (13), Burnett and Jones (4)), but Valteau and Johnson (12) have produced streak symptoms by inoculation of the latent potato virus together with three strains of tobacco mosaic, three strains of cucumber mosaic, three strains of etch, as well as with an unmixed virus belonging to the tobacco mosaic group, ring mosaic.

Streak is of rare occurrence in glasshouses in this country.

The disease may be readily synthesised and analysed. Either tomato mosaic or glasshouse streak when mixed with potato mosaic causes streak. When *Datura Stramonium* (or *Nicotiana glutinosa*) is inoculated with streak local lesions form, typical of the tomato mosaic or glasshouse streak component, while potato mosaic alone becomes systemic causing vein necrosis and mottle and may be isolated in a pure state (see Plate XXIV, fig. 6). If juice from a streaked plant is stored *in vitro* the potato mosaic loses its activity after 6 months, when the tomato mosaic or glasshouse streak component which does not age so quickly may be obtained free from potato mosaic. Other differences between streak and glasshouse streak are that the former almost invariably gives 100 per cent. of streaked plants on inoculation—glasshouse streak is much more variable in this respect, see Table III—and the incubation period (using young tomato plants) is 5–7 days under the most favourable summer conditions and only a day or two longer in winter when the incubation period for tomato mosaic is about 3 weeks. Working with young plants streak rarely gives a mottle only, and when it does plants inoculated from such a plant show tomato mosaic symptoms only.

There is no difference in symptoms on tomato whether tomato mosaic or glasshouse streak is combined with potato mosaic, but the two mixtures can be distinguished by tobacco (*N. tabacum*) inoculations. In both cases local lesions are produced. With a tomato mosaic mixture a severe necrosis of the leaves above those inoculated follows, while with a glasshouse streak mixture, though some plants show a severe leaf necrosis, others only show systemic symptoms due to potato mosaic. That the components of streak remain distinct for long periods is demonstrated by the following example. A glasshouse streak-potato mosaic streak was inoculated into tobacco after fifteen consecutive transfers (during 9 months) on tomato when the potato mosaic at once separated out. Tobacco plants inoculated with dried tomato tissue, infected with the same streak, after 10 months' storage showed only glasshouse streak symptoms.

SPOTTED WILT.

Glasshouse streak and streak have in the past been confused with spotted wilt. Spotted wilt was first described in Australia in 1919 by Brittlebank (3) and first recorded in England last year (1931). During the past season plants suffering from this disease have been received at Cheshunt from growers in different parts of the country, and this afforded an opportunity to compare the diseases. Spotted wilt was found to be

quite distinct from glasshouse streak and streak. Adequate descriptions of spotted wilt have already been published (see Samuel *et al.* (10), Smith (11), Ainsworth (1)) so no detailed account will be given here.

Table IV summarises the differences between tomato mosaic, glasshouse streak, streak and spotted wilt.

Table IV.

Comparison of tomato mosaic, glasshouse streak, streak and spotted wilt.

	Tomato mosaic	Glasshouse streak (single virus)	Streak (mixed virus)	Spotted wilt
Filterability	Can be filtered through	Pasteur-Chamberland filters		Cannot be filtered
Longevity <i>in vitro</i>	Several years	Several years	Six months, after which potato mosaic component inactive	A few hours
Differential hosts:				
1. <i>Lycopersicum esculentum</i>	Mottle, slight to severe leaf distortion, no necrosis. Fruit not marked	Mottle and/or necrotic stripes on stem and petioles with necrotic areas on leaves. Fruit marked	As glasshouse streak but mottle only rare. Fruit marked	Bronzing of leaves, stunting, yellow mottle. Necrosis of stem or petioles rare. Fruit marked
2. <i>Nicotiana tabacum</i>	Mottle and leaf distortion; no necrosis	Necrotic local lesions, systemic necrosis and mottle	See p. 426	Large zoned necrotic local lesions, severe systemic necrosis
3. <i>Nicotiana glutinosa</i>	Necrotic local lesions, no systemic infection		Potato mosaic component only becomes systemic	Large necrotic local lesions, systemic infection usually kills plant
4. <i>Datura Stramonium</i>				Local lesions in form of rings, systemic infection

SUMMARY.

Virus diseases of tomato of the mosaic, "stripe," streak type as occurring in glasshouses in the British Isles have been examined.

Ordinary or mild mosaic (tomato mosaic) has been identified with true tobacco mosaic and found not to be implicated in most of the "stripe" disease.

The symptom picture generally known as "stripe" may be due to several causes, and in this paper the diseases have been distinguished as follows: *stripe* when attributed to *Bacillus lathyri*, *glasshouse streak* when caused by a single virus (the most frequently occurring form), and *streak* when due to a mixed virus infection (typically tomato mosaic and potato mosaic).

Descriptions are given of tomato mosaic, glasshouse streak, and streak, and they are compared with each other and with spotted wilt.

The author wishes to express his best thanks to Dr W. F. Bewley for helpful criticism and advice.

The work has been carried out with the aid of a grant from the Empire Marketing Board.

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EXPLANATION OF PLATES XXIII AND XXIV.

PLATE XXIII.

- Fig. 1. Tomato (var. E.S. 1) affected with glasshouse streak.
- Fig. 2. *Nicotiana tabacum*, 3 weeks after inoculation with tomato mosaic.
- Fig. 3. *Nicotiana tabacum*, 13 days after inoculation with glasshouse streak. *a*, primary systemic necrosis; *b*, necrotic local lesions only.
- Fig. 4. *Nicotiana tabacum*, 5 weeks after inoculation with glasshouse streak. Later stage of primary systemic necrosis.
- Fig. 5. *Nicotiana tabacum*, 5 weeks after inoculation with glasshouse streak; secondary systemic necrosis.

PLATE XXIV.

- Fig. 6. *Datura Stramonium*, 9 days after inoculation with streak. Note necrotic local lesions on the left-hand leaf due to the tomato mosaic and the early potato mosaic symptoms towards the base of that leaf and on other leaves.
- Fig. 7. *Solanum melongena*, 2 weeks after inoculation with glasshouse streak.
- Fig. 8. *Solanum melongena*, 2 weeks after inoculation with tomato mosaic.

(Received December 31st, 1932.)



Fig. 1.



Fig. 2.



Fig. 3.



Fig. 5.



Fig. 4.

AINSWORTH.—AN INVESTIGATION OF TOMATO VIRUS DISEASES OF THE MOSAIC "STRIPE,"
STREAK GROUP (pp. 421-428).



Fig. 6.



Fig. 7.



Fig. 8.

AINSWORTH.—AN INVESTIGATION OF TOMATO VIRUS DISEASES OF THE MOSAIC "STRIPE,"
STREAK GROUP (pp. 421-428).

ON THE BIOLOGY OF THE PLUM SAWFLY, *HOPLOCAMPA FLAVA* L.¹, WITH NOTES ON CONTROL EXPERIMENTS

BY F. R. PETHERBRIDGE, I. THOMAS AND G. L. HEY.

(School of Agriculture, Cambridge.)

(With Plate XXV.)

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I. INTRODUCTION.

INJURY to young plums from the tunnellings of sawfly larvae has been known in this country for a long time. In 1891 Ormerod⁽⁴⁾ records this damage from Northants, Gloucestershire and Worcestershire. She does not attempt to identify this pest other than to say: "I have only had opportunity of studying in larval state; therefore I have merely distinguished it at heading by the name of Plum Sawfly." There is nothing in her description of the damage which differs from our observations on *H. flava* L.

In his *Insect Pests of Fruit* published in 1909, Theobald⁽⁶⁾ states: "The plum has long been observed by growers and gardeners to be attacked by a large maggot, which we now know to be the *Hoplocampa fulvicornis* of Klug, the *Tenthredo morio* of Schmidberger and Kollar." Theobald makes no mention that he bred out or collected adult sawflies and apparently based his identification on the larvae and the damage caused by them. His description of the adult agrees with that of *H. minuta* Christ. (= *H. fulvicornis* F.), of which species there is no record

¹ The morphology of this species will be described in a subsequent paper.

in England. Sprengel⁽⁵⁾ includes Theobald's record under *H. minuta*. Mr R. B. Benson of the British Museum (*in litt.*) says: "I have never seen British caught specimens of *H. minuta*, nor has Dr Perkins whom I questioned on the point."

From the fact that Theobald was dealing with a species which caused serious injury to plums and that there is still only one species in this country which does serious damage, it seems clear that he was mistaken in his identification and that the larvae he found in plums were undoubtedly those of *H. flava* L. Many observers in this country have followed Theobald in attributing plum sawfly damage to *H. fulvicornis* Klug.

The first record of *H. flava* L. damaging plums in this country was made by us in April, 1926 (*Monthly Report to the Ministry of Agriculture for April*, 1926). The identification of the species was made by Morice at the British Museum. Warburton's⁽⁷⁾ record was based on this identification. Since that date we have collected large numbers of adults chiefly by beating plum branches on to a beating tray, and in all cases, with one exception¹, the species we obtained was *H. flava* L.

From this it seems almost certain that all records of plum sawfly damage in England referred to *H. fulvicornis* Klug should be referred to *H. flava* L.

A full account of the synonymy of *H. flava* L. and *H. minuta* Christ., the two species causing damage to plums on the Continent, is given by Sprengel⁽⁵⁾, who also deals with the biology and morphology of these species on plums in Germany. In the main our observations in East Anglia are in agreement.

H. flava L. has also been recorded as damaging plums in France⁽¹⁾ and Italy⁽²⁾.

II. BIOLOGY.

(a) *The adult.*

The sexes differ somewhat in appearance. The female, which is the larger, has an orange-coloured thorax with black areas at the bases of the wings and along the edges of the metanotum. The eyes and ocelli are black, and the antennae, abdomen and legs are yellowish orange. In the male the thorax is darker than in the female, the black areas extending to the scutellum and edges of the scutum. In other respects the colouring of the sexes is the same.

¹ The exception has been identified by Dr H. W. Miles as *H. chrysorrhoea* Klug.

The adults emerge from the soil about a week before Czar plums are in full bloom, the emergence usually coinciding with the opening of the blossoms, which varies considerably from season to season (see Table II). On emergence they either crawl over the ground and up the trunks of the trees or fly directly on to the blossoms. If shaken from the trees in dull weather they crawl sluggishly over the ground until they come to a tree which they ascend; in sunny weather, however, they fly readily back to the blossoms. The adults live about 14 days after emergence, the males being somewhat shorter lived than the females.

In 1926 the following observations were made in order to determine whether any correlation exists between the dates of emergence of the adults and the weather conditions, and also to compare these dates with the date of flowering of the plum. For this purpose a plantation of Czar plums growing on a well-drained rich sandy loam at Cottenham near Cambridge was utilised. Early in March seventy-two cages of lath and muslin, about a foot high, were placed on the soil under the plum trees in such a way that any sawflies emerging from the soil were trapped. Records were kept of the weather conditions and ground temperatures during the period under observation. The erection of the cages was completed on March 19th, and at this time the blossom buds of the Czar plums were swelling rapidly. The season was a very early one, the last week in March being mild and dry. On March 28th, the blossoms began to open, and on April 5th the trees were in full bloom, an unusually early date (see Table II).

Table I.

*Showing the number of H. flava emerged in cages
during March and April, 1926.*

Date	Temperature at ground level at 9 a.m. ° F.	Number of sawflies emerged
March 30	36	0
" 31	38	1
April 1	38	1
" 2	36	3
" 3	46	5
" 4	51	6
" 5	45	4
" 6	40	10
" 7	41	0
" 8	42	2
" 9	38	2
" 10	38	2
" 11	40	0
" 12	42	1
" 13	45	0

The cages were examined daily for the presence of sawflies and the first appeared on March 31st, by far the earliest date we have ever recorded. Table I gives particulars of the emergence.

This table shows that the temperature at ground level rose steadily until April 4th, and the curve of emergence of the sawflies follows the temperature curve and reaches its maximum on April 6th, two days later. Nearly 70 per cent. of the total emergence took place from April 3rd to April 6th. Nearly half an inch of rain fell on April 7th and no sawflies emerged that day, but on the following three days they continued to emerge, and the last one emerged on April 12th. Subsequent observations show that the season 1926 was exceptionally early, and it is probable that the period of emergence is longer than would obtain in a normal season.

In 1930 we grease-banded sixty plum trees before the sawflies had emerged and on these we caught seventeen adults. Later on it was noticed that these trees were badly infested with sawfly larvae. From this it would appear that most of the sawflies must have flown on to the trees, either directly from the ground or from neighbouring trees.

In order to study their habits, adults were collected in the orchard and kept on plum shoots in cages in the laboratory. In 1932, seventy-two adults were collected by shaking the branches of plum trees over a beating tray, and of these sixty-three were females. In the cages the females were very active, crawling or making partly jumping and partly flying excursions from blossom to blossom. This activity was not noticeable in the orchard unless they were disturbed. The males remained hidden among the blossoms and were often very difficult to detect because of their resemblance to the dead scales. Both sexes, and especially the females, fed extensively on the nectar and pollen of the flowers.

Fertilisation appears to occur shortly after emergence, for a few adults were taken *in coitu* on the ground, on the tree trunks, or trapped on the grease-bands. In the laboratory it was observed that one male fertilised more than one female.

In one case a male, after remaining *in coitu* with a female for 5 min., immediately fertilised another female, remaining *in coitu* for about 4 min. When copulation takes place the abdomens of the male and female are in a straight line and the apices of the male's wings are placed over those of the female.

(b) *Oviposition.*

In the laboratory eggs were laid about 2-3 days after fertilisation, and at this time most of the flowers were fully open or beginning to turn brown. Eggs were occasionally laid in flowers which had just opened. When egg-laying the female faces down the stem of the flower, clasping the receptacle with her legs. She makes a small incision with her ovipositor in the receptacle and in this she deposits an egg. The position of the egg can be seen from the outside by the jagged elongated puncture which has brown margins (Plate XXV, fig. 1). Only one egg is laid in each flower and the process occupies from $\frac{1}{2}$ to 3 min. The exact position of the egg varies, but it is usually laid in the wall of the receptacle. Sometimes, however, it is to be found in the tissue of one of the sepals and occasionally it may be found loose among the stamens.

The number of eggs laid by one individual was not determined, but females examined before oviposition had commenced contained up to fifty developed eggs in addition to immature ones.

Table II.

Showing in detail observations made on the time of the first appearance of the adults and the time of egg-laying as compared with the date of flowering and also with the date on which the first larvae were found.

Year	First appearance of adults	Czars in full bloom	Egg-laying commenced	Last date adults caught	First larvae found	First cocoons spun
1926	March 31st	April 5th	—	—	April 27th	—
1927	—	—	—	—	—	June 27th
1929	—	May 4th	—	—	—	June 11th
1930	April 24th	April 30th	May 3rd	May 10th	May 18th	—
1931	April 22nd	April 30th	April 29th	May 4th*	May 15th	June 16th
1932	April 24th	May 7th	April 30th	May 14th	May 21st	June 24th

* No observations were made between May 4th when the last sawflies were found and May 15th when no sawflies were found.

(c) *The larva.*

On hatching the young larvae are about 1.6 mm. in length and soon begin to bore into the young developing fruitlets, which in the case of Czar plums are about 5 mm. long at this date (Plate XXV, fig. 2). The withered receptacle and its floral appendages remain for a time surrounding the fruitlets and cover the larval entrance hole, which is usually situated about two-thirds of the distance from the base to the apex of the plum, but it may be found in almost any position.

Table III gives our observations on the duration of the five instars as compared with Sprengel's (5) observations in Germany.

Table III.

Instar	Our observation days	Sprengel's observation days
1	9	9
2	6	7
3	6	4
4	6	3-4
5	6	3

In the second instar the larva usually migrates to another plum and soon its tunnelling becomes very obvious on account of the blackish frass which is exuded from the entrance hole. A characteristic odour resembling that of benzaldehyde is also given off. Inside the plum the larva lies curled up, surrounded by black frass, in the tunnel it has made.

There is a second moult about 15 days after hatching when Czar plums measure from 5 to 12 mm. in length and the receptacle and its appendages have mostly dropped. In each plum there is usually one hole and one larva, but occasionally two holes may be found and two larvae may be present. In one case there were three larvae present in a plum with two holes.

In the third and succeeding instars the larvae feed on the stone, a portion or all of which may be eaten and the centre of the plum filled with black frass. Further migration occurs in the fourth and fifth instars, and one larva may feed on as many as four fruitlets.

When migrating the larvae crawl out backwards from the tunnel and sometimes bite small pieces from one or more plums before finally making an entrance into another plum. About 30-35 days after hatching they are fully fed and are now from 9 to 11 mm. long. They now back out of the fruit and drop to the ground, into which they start burrowing immediately. Nearly all the damaged fruits fall from the tree before they are ripe, but occasionally ripe fruit with tunnels, but no larvae may be found. A small porportion of the larvae die inside the plums, and in a large percentage of these cases the flesh of the fruit and the frass becomes wet and glue-like. This was more noticeable in Pond's Seedling than in any other variety.

(d) *The cocoon.*

Soon after entering the soil the larva constructs a cocoon. This measures about 5.5 mm. in length and about 2.5 mm. in width. It is made of a brown parchment-like material and its outer appearance depends on the texture of the soil in which it is formed. When formed in a sandy soil the sand particles adhere to its surface and give it a rough-coated appearance. When formed in a clay soil the cocoon is extremely

difficult to find, as it is then smooth coated and usually hidden inside a lump of clay.

In order to find out the depth at which pupation takes place, two drain pipes, one above the other, were sunk in the ground and filled with soil so that its level inside the pipe after compacting was the same as the soil level. A large number of larvae and plums containing larvae were placed on top of the soil in the drain pipes. At the end of July the soil from the drain pipes was removed and sifted. Only twelve cocoons were recovered, and of these none were found in the top 2 in.; four were found in the next 3 in.; seven in the next 4 in. and one in the next 2 in. No cocoons were found below 11 in. This is in close agreement with Miles' (3) finding in the case of *H. testudinea* Klug. The larvae live throughout the winter and some of them pupate in the spring about 3 weeks before the emergence of the adults. Some larvae are capable of remaining as such for a longer period. Three drain pipes in which larvae had formed cocoons in June, 1931, were sunk in the soil under plum trees, and cages made from wired cellophane were constructed to cover the top of each drain pipe and to include a small branch from a plum tree. No sawflies came out of these pipes. On June 27th, 1932, the soil from these drain pipes was examined and fourteen cocoons recovered. Of these, six contained viable larvae; six contained shrivelled remains, and two contained dead adults. In the two latter a second cocoon had been constructed inside the first. From the above it would appear that this species possesses the characteristic of delayed development, as it seems probable that larvae¹ remain in the cocoons over a second winter before pupating. It is not known what proportion exhibit this phenomenon or what factors influence this proportion, but it is possible that these influence the severity of the infestation in any one season and are a safeguard to the persistence of the species.

Table IV.

Showing the extent of damage by H. flava over a period of years.

Year	Percentage of fruitlets damaged
1925	Over 50 %*
1926	Below 10 %
1927	25-30 %
1928	Below 10 %
1929	10-15 %
1930	Below 10 %
1931	10-15 %
1932	40-50 %

* Some growers estimated their loss at 90 % of the crop.

¹ Some of these larvae were found to be parasitised, the parasites emerged in 1933.

Observations in plantations in the Eastern Counties show that the plum sawfly is an exceptionally variable pest and that a severe outbreak in one season may be followed by a mild attack during the following one. In this connection observations made in a plantation of Czar plums growing on a well-drained rich sandy loam at Cottenham, near Cambridge, are of interest. These are shown in Table IV.

The following note written in June, 1891, by Mr W. F. Gibbon of Evesham to Miss Ormerod(4) shows a similar fluctuation: "Last year I noticed a lot of them (infested plums) and had all the dropped plums daily gathered up and burnt. This year I find a bored plum dropped only here and there."

(e) *Variation in susceptibility of varieties.*

In the plum plantations of Cambridgeshire and Huntingdonshire, where we have made most of our observations, the variety which has suffered most from sawfly attacks is the Czar plum. Next to this in intensity of attack comes the Victoria. The varieties which have suffered least are Monarch and Pond's Seedling.

We suggest that the time of flowering of a variety may play a part in the intensity of the attack. The Czar plum flowers in *mid-season* and the Victoria flowers about the same time. Monarch flowers much earlier (about 7 days) than Czar, and at the time of egg-laying its flowers are much more advanced than those of varieties which flower in mid-season, and are probably not so suitable for egg-laying. On the other hand Pond's Seedling flowers much later (about 7 days) than Czar, and at the time of egg-laying is not nearly so advanced as those varieties which flower in mid-season and probably not so suitable for egg-laying at the time when oviposition is at its maximum. In this connection it must be remembered that adults only live about 14 days, and the longest period over which we have found adults is 20 days. This was in 1932. The period was probably longer than this in 1926, as in that season they emerged from the soil over a period of 13 days.

III. CONTROL EXPERIMENTS.

Sprengel(5) has carried out a number of experiments on the control of this pest. She states that there are three possible methods of controlling the larva:

- (1) The use of lead arsenate,
- (2) The use of nicotine sulphate in addition to lead arsenate,¹
- (3) The use of nicotine in addition to lead arsenate,

¹ In 1933 results sufficiently good to be of economic value have been obtained by two applications of a spray consisting of lead arsenate, nicotine sulphate and a spreader.

and she concludes by stating that a good control may be obtained by spraying with an arsenical 8 days after petal fall and again 8 days later. The efficiency of this spray is increased by the addition of nicotine sulphate.

In 1926 the soil beneath the trees of a Czar plum plantation at Cottenham was given a heavy application of gas lime, but practically no reduction of the damage done was noticeable as a result of this.

In 1929 an experiment was carried out on the same plantation to test the effect of a spray consisting of soft soap 4 lb., nicotine 4 oz., water 40 gallons. (Owing to the hardness of the water an extra 2 lb. of soft soap was added for the purpose of softening the water.) The trees sprayed were Czar plums, and they were in full bloom on May 4th. The first plot was sprayed on May 3rd and the second plot on May 10th. The spraying was done by means of a barrow hand-pump. A number of plums from sprayed and unsprayed trees were examined and the number of damaged plums counted. The results obtained are shown in Table V.

Table V.
*Showing the percentage of damaged plums from
sprayed and unsprayed trees in 1929.*

Date of spraying	No. of trees	No. of fruitlets examined	No. of fruitlets damaged	% damage
May 3rd	5	1110	27	2.4
May 10th	4	821	15	1.8
Unsprayed	5	944	107	11.3

In 1932 these experiments were repeated on Czar plums in Cambridgeshire and Huntingdonshire. The plums were in full bloom on May 7th, and similar spraying to the above was carried out on May 18th and May 21st. Counts of the percentage of damaged plums showed that there was no significant difference between the sprayed and unsprayed trees.

The percentage infestation of the control trees was 45.5 per cent. in Cambridgeshire and 13.3 per cent. in Huntingdonshire.

It is difficult to explain why promising results were obtained in 1929 and not in 1932. From Sprengel's results it would appear that our sprayings were made too early.

IV. SUMMARY.

Observations on the biology of *Hoplocampa flava* L. are given.

Reasons are given for stating that all records of plum sawfly damage in England referred to *H. fulvicornis* should be referred to *H. flava* L.

Observations are given showing the variation in intensity of attack during 1925-32.

Control experiments with the object of killing the larvae were promising in 1929, but yielded no control in 1932.

The authors are indebted to Dr H. W. Miles and Mr H. Kingston, who were associated with us in the above observations in 1926, and to Messrs M. H. Ivatt and G. H. Cannon, on whose plantations the observations were made.

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EXPLANATION OF PLATE XXV.

Fig. 1. Site of oviposition of *H. flava*.

Fig. 2. Young Czar plum showing entrance hole of first instar larva and scar where an entrance was not effected.

(Received November 19th, 1932.)

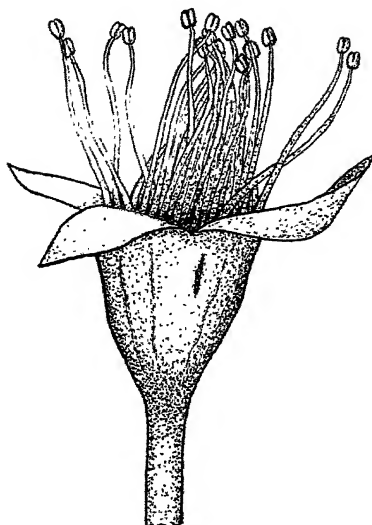


Fig. 1.



Fig. 2.

PETHERBRIDGE, THOMAS AND HEY.—ON THE BIOLOGY OF THE PLUM SAWFLY, *HOPLOCAMPA FLAVA* L., WITH NOTES ON CONTROL EXPERIMENTS (pp. 429-438).

ON THE BIOLOGY OF *CACOEZIA CRATAEGANA* HUB. (LEPIDOPTERA: TORTRICIDAE) ON FRUIT TREES IN THE WISBECH AREA

BY G. L. HEY AND I. THOMAS.

(School of Agriculture, Cambridge.)

(With Plates XXVI and XXVII and 13 Text-figures.)

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I. INTRODUCTION.

THE first record of *Cacoecia crataegana* Hub. in Great Britain appears to be that of Wood (25) in 1854. He figures the moth under the synonym of *Lozotaenia roborana*, the Oak Red Bar, and records it as having been taken at Darenth Wood, and occasionally near London.

The Entomological Societies of Oxford and Cambridge in 1858 record the moth in their *Accentuated List of British Lepidoptera* (6) as *Tortrix crataegana* Hub. They state that it is "named from the hawthorn (*Crataegus*); but the larvae feed on fruit trees." This seems to be the only instance where fruit trees are given as host plants in Great Britain,

though there are several foreign records of the larva on fruit. Other records are those of Stainton⁽²¹⁾, Wilkinson⁽²⁴⁾, Morris⁽¹⁷⁾, Meyrick^(14, 15), Barrett⁽¹⁾, and Hofmann⁽¹⁰⁾; the recorded food plants of the larva being oak, birch, willow, hawthorn, blackthorn, aspen, poplar, medlar, cotoneaster and apple. A record of the larva being injurious to plum and apricot in Astrakhan is given by Sacharov⁽¹⁸⁾, while Mokrzechi⁽¹⁶⁾ lists it as a pest of fruit trees in the Crimea. In the Konchû Sek, Gifu⁽¹¹⁾ it is recorded amongst the noxious insects of mulberry trees in the region of Iga, Japan.

A short account of the earlier stages of *C. crataegana* Hub. is given by Sheldon⁽²⁰⁾. As host plants he notes oak, elm and willow. The most recent record is that of Hesse⁽⁹⁾, on *Lilium martagon*, in Germany.

The presence of the curious egg-masses of this moth on plum and apple in a large orchard near Wisbech was brought to the notice of Mr F. R. Petherbridge in January, 1930. On the establishment of the identity of the moth, Mr J. C. F. Fryer, in correspondence with Mr Petherbridge, stated: "*C. crataegana* as a pest is quite new to me. It is not a very common insect, and I have usually reared it from larvae found on willows growing in woods." As the larvae were feeding on fruit trees, it was decided to investigate its life history and give a description of its immature stages. In Nova Scotia⁽¹⁹⁾, a closely allied moth, *C. rosaciana* Harris, the Oblique-banded Leaf Roller, is known to be a widely distributed pest of apples, while *C. argyrospila* is one of the injurious Fruit Tree Rollers of U.S.A.⁽²³⁾ and British Columbia⁽⁵⁾.

II. DESCRIPTION OF STAGES AND BIOLOGY.

(a) *The egg and egg-laying.*

As far as can be ascertained, there is no description of, or even reference to, the egg of *C. crataegana* in the literature. The egg-masses of a closely related species, *C. argyrospila*, which are very similar in appearance, except that they are brown in colour, are figured by Vincent⁽²³⁾ in U.S.A. and by Eastham and Ruhmann⁽⁵⁾ in British Columbia.

About thirty eggs are laid together to form a white egg-mass (Plate XXVI, fig. 1). Each egg is somewhat cylindrical in shape and measures about 0.64 by 0.37 mm. They are laid in honeycomb formation with their bases towards the bark of the tree (Plate XXVI, fig. 2). The individual eggs are cemented together by means of a white, brittle, opaque, organic material. On analysis this material was found to consist

of: moisture (lost at 100°C .) 2.8 per cent., organic matter 90 per cent., and inorganic matter 7.2 per cent. This latter consisted of Fe_2O_3 1.9 per cent., CaCO_3 2.0 per cent., NaCl 3.3 per cent. and traces of P_2O_5 and SO_3 . A very thin layer of this material is present between the base of the eggs and the bark. It is also continued around the edges of the mass and extends over the top of the eggs in a thicker layer, varying from 0.1 mm. in thickness at the edges to 0.21 mm. in the centre (Plate XXVI, fig. 3). The eggs are thus completely enclosed by this cement-like material to form an irregular-shaped egg-mass measuring about 4.59 by 2.55 mm., and varying from 3.55 to 5.55 mm. in length, and 1.99 to 3.1 mm. in breadth.

These egg-masses closely resemble specks of lime on the trees. Some years previously the trees where they were first noticed (Plate XXVI, fig. 4) had received a dressing of lime-wash and where this had persisted the unhatched egg-masses could not, at some distance from the trees, be distinguished from specks of lime. The pale yellow eggs have very thin walls, which are somewhat flattened where the eggs are pressed together in brick-like formation in the centre of the egg-mass (Plate XXVI, fig. 5). The eggs were first noted in the field on July 7th. They are laid in the slight depressions of the bark, on the upper part of the trunk and on the main branches of the tree. Egg-laying continues throughout July and August. In cages in the laboratory a female was seen ovipositing on the wooden framework on the evening of July 6th.

(b) *Hatching.*

The insect over-winters in the egg stage. In 1930 the first eggs hatched on May 1st and in 1931 on April 15th. The first indication of hatching is the appearance of a mandible of the larva through the surface of the egg-mass. The larva then makes a small hole and bites round the edges until the aperture is big enough for the head to emerge. Some pieces of the organic matter are pushed upwards out of the hole, and other pieces are eaten by the larva. Occasionally some of these get entangled on the maxillary setae and the larva then finds it difficult to get them into its mouth. Whilst biting its way out of the egg-mass, the body of the larva does not turn round inside the egg, and more material is bitten off the egg-mass near the sides than off that part either behind or in front of the head. In this manner the hole assumes an almost elliptical shape (Plate XXVI, fig. 6). When the hole is big enough, the larva thrusts out its head, and by wriggling from side to side the thoracic segments follow almost immediately. After resting for a few seconds it

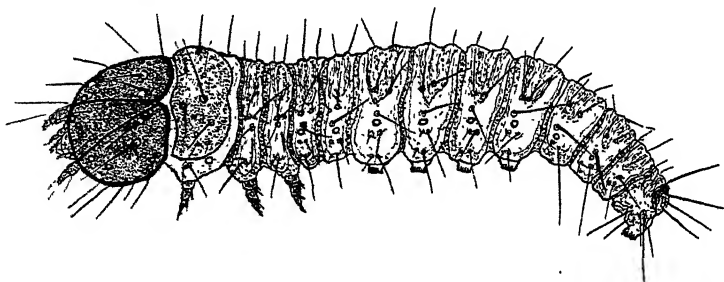
places its thoracic legs on the surface of the egg-mass and pulls itself out.

The process of biting the hole occupies from 30 to 40 min., whereas the actual withdrawal of the larva from the egg takes only 20–60 sec. After defaecating on the egg-mass, and in some cases after cleaning its mouth-parts, the larva crawls rapidly towards the light. The empty egg-masses are cribiform (Plate XXVI, fig. 6) and may remain on the tree for several years, so that fresh ones are often partially imposed on them.

(c) *The larva.*

(1) *First instar.*

The first stage larva (Text-fig. 1) is very pale green in colour, about 1.41 mm. long, with the thoracic segments slightly darker than the



Text-fig. 1. First stage larva ($\times 54$).

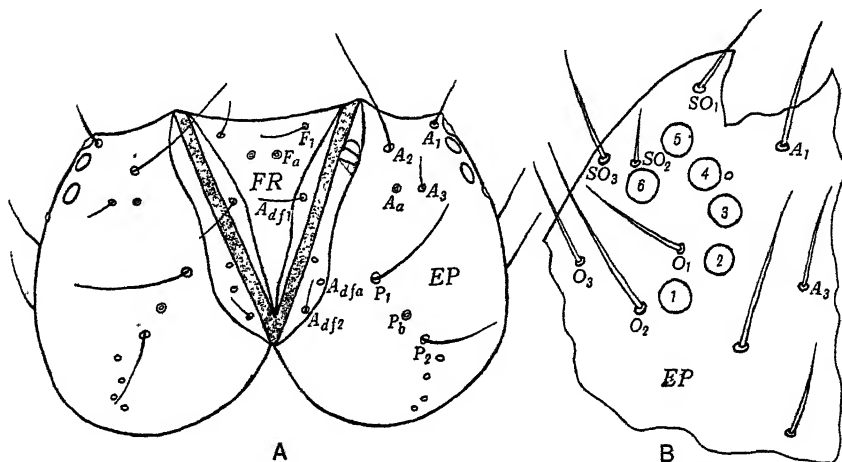
abdominal. Small, black, lightly chitinised areas around the base of each seta, or group of setae, give the body a mottled appearance.

The head. The head capsule (Text-fig. 2) is shiny black, 0.274 mm. in width, and strongly chitinised. It consists of a pair of *epicrania* (*EP*), between which, where they extend to the dorsal surface, lies the V-shaped *front* (*FR*). The latter is attached to the epicranium on either side by a long, narrow, *adfrontal* (*AD*) which extends forward as far as the *clypeus*.

The ventral surface is membranous, with the exception of two small triangular sclerites, the *post-genae*.

The ocelli are six in number; the first five form on the dorsal surface a quadrant of a circle, with numbers 3, 4 and 5 closer together. Number 6 lies on the ventral surface opposite number 4. The epicranium has the normal number of setae and punctures. The anterior setae A_1 , A_2 , and A_3 form an isosceles triangle with A_2 at the apex. The anterior puncture A_a

is immediately posterior to A_2 . The ocellar setae O_1 , O_2 and O_3 lie behind the ocelli; O_1 is equidistant from ocelli 1 and 2, O_2 is immediately behind ocellus 1, while O_3 is remote and lies on the ventral surface almost equidistant from ocelli 1 and 6. The sub-ocellar setae SO_1 , SO_2 and SO_3 form an obtuse angle. SO_2 lies immediately below ocellus 6, with SO_3 a short distance away, and SO_1 lies in the ventral anterior angle of the epicranium, a short distance in front of ocellus 5. The posterior setae P_1 , P_2 and the puncture P_b lie in a straight line. There are four small ultra-posterior punctures. The adfrontal seta A_{df2} lies at the level of



Text-fig. 2. A, head capsule of first stage larva ($\times 235$). B, portion of epicranium of fifth stage larva ($\times 235$). *AD*, adfrontal sclerite. *EP*, epicranium. *FR*, front. A_1 , A_2 , A_3 = anterior setae; A_a = anterior puncture; A_{df1} , A_{df2} = adfrontal setae; A_{dfa} = adfrontal puncture; P_1 , P_2 = posterior setae; P_b = posterior puncture; F_1 = frontal seta; F_a = frontal puncture. 1, 2, 3, 4, 5 and 6, ocelli. *O* = ocellar setae; *SO* = sub-ocellar setae.

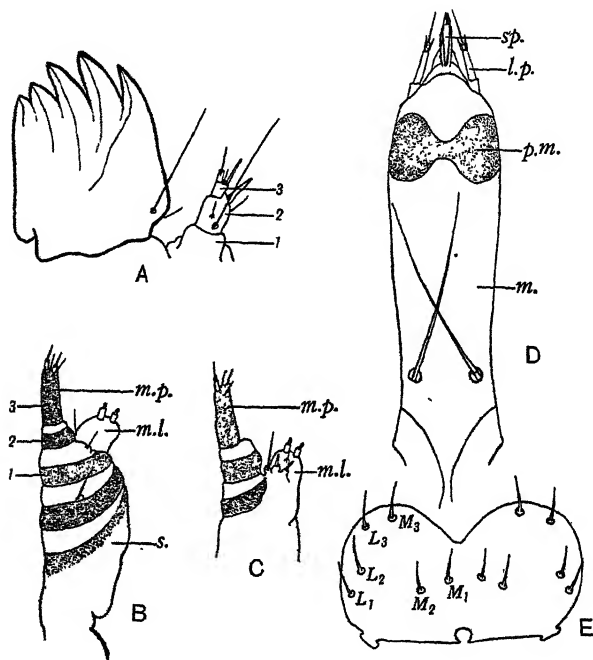
the apex of the front, and the seta A_{df1} lies midway between the anterior margin and the apex. The frontal seta F_1 lies near the anterior margin of the front with the frontal puncture F_a in a position slightly posterior and near the median line.

The *labrum* (Text-fig. 3 E) has a deep median incision; the setae L_1 and L_2 lie together near the lateral margin, L_3 and M_3 lie near the anterior margin, while M_1 lies near the median line midway between the anterior and posterior margins, with M_2 in a position slightly removed laterally and posteriorly.

The *antennae* (Text-fig. 3 A) are very short, three-jointed with the second joint slightly longer than the first. A long seta arises near the

base of the second joint of the dorsal surface, while just in front of it is an extremely fine bristle. A large papilla and a fine bristle are also present on the ventral surface. The third joint bears a short fine bristle, an elongated papilla, and a small papilla terminating in a fine seta.

The mouth-parts. The *mandibles* (Text-fig. 3 A) are alike, sub-quadrate and five-toothed, each bearing a long seta on the dorsal surface near its



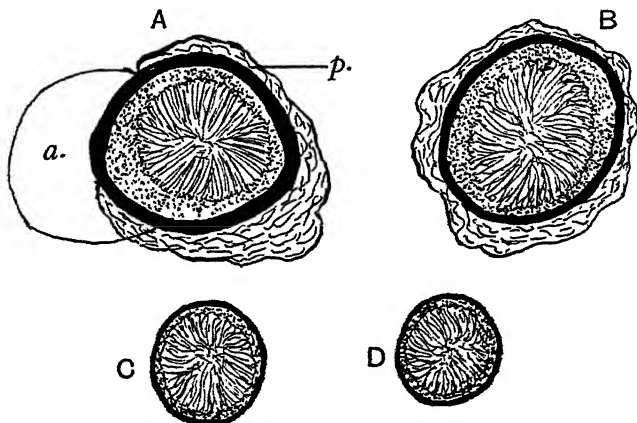
Text-fig. 3. Mouth-parts of first instar larva ($\times 320$). A, right mandible and antenna (dorsal). B, right maxilla (ventral). C, left maxilla (ventral). D, labium (ventral). E, labrum (dorsal). *l.p.* = labial palp; *m.* = mentum; *m.l.* = maxillary lobe; *m.p.* = maxillary palp; *p.m.* = prementum; *s.* = stipes; *sp.* = spinneret. *L* and *M* = setae.

latero-posterior margin, and in a similar position on the ventral surface, a short fine bristle.

The *maxillae* (Text-fig. 3 B and C) consist of *cardo* and *stipes* (*s.*); the two-jointed *maxillary palp* (*m.p.*) of the latter terminates in two bristle-bearing papillae and three short setae. Situated on its lateral margin adjacent to the labium is a short *maxillary lobe* (*m.l.*). At its apex are two short papillae, both two-jointed, and in a position median and posterior to these is a short papilla bearing a very fine bristle. Immediately behind this papilla are two larger papillae each terminating

in a short thick seta. On the lateral margin of the lobe adjacent to the maxillary palp is a third papilla bearing a long bristle. The stipes bears on its ventral surface two setae, one adjacent to the base of the maxillary lobe, and another almost in the centre of the stipes.

The *labium* (Text-fig. 3 D) occupies the ventral portion of the head between the maxillae. It consists of a lightly chitinised *mentum* (*m.*) and a *prementum* (*p.m.*) which carries the *spinneret* (*sp.*). Two very long setae arise near the posterior margin of the mentum on the ventral surface. The *labial palps* (*l.p.*) lie one on either side of the spinneret. Each consists of a short thick basal joint and an attenuated second joint. From the latter arise two short setae and between these a two-jointed



Text-fig. 4. Spiracles of fifth instar larva ($\times 170$). A, prothoracic spiracle. B, eighth abdominal spiracle. C, seventh abdominal spiracle. D, third abdominal spiracle. *a.* = atrium; *p.* = peritreme.

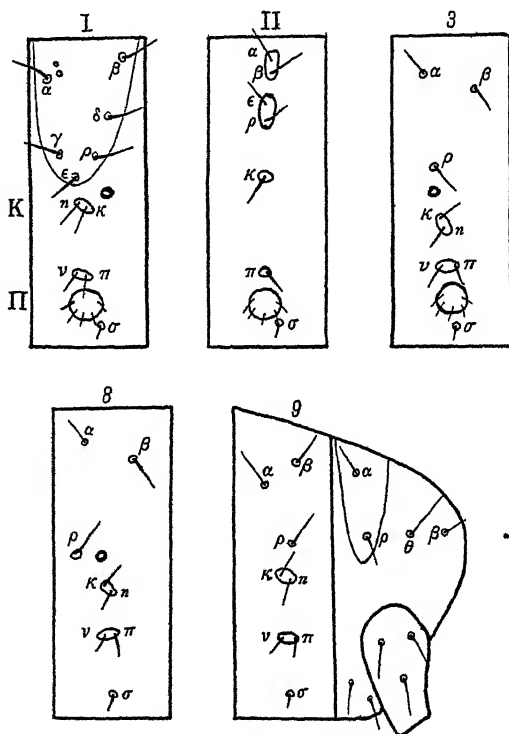
palpus bearing a thin bristle. The spinneret itself terminates in two minute papillae, both two-jointed.

The *body* (Text-fig. 1). The *prothoracic shield* is large, black, and strongly sclerotised. The *anal plate* in this stage is extremely narrow, black, but not heavily sclerotised. Under a high magnification the whole chitinous covering of the body is seen to consist of minute tubercles, from each of which arises a microtricha.

The *spiracles*. There is a pair of *spiracles* (Text-fig. 4) on the prothoracic, and a pair on each of the first eight abdominal segments. They are typical of a *Tortrix* larva, each being surrounded by a thick circular *peritreme* (*p.*) from which arises a series of processes beneath which is the single-chambered *atrium* (*a.*).

The thoracic spiracles are large, heavily sclerotised, and are situated immediately below the cephalic plate nearer the posterior than the anterior margin of the segment.

The first six abdominal spiracles are small and of equal size, they are not as heavily chitinised as the thoracic spiracle and are situated nearer the anterior than the posterior margin of each segment. The



Text-fig. 5. Setal map of first instar larva. I, first thoracic segment. II, second thoracic segment. 3, third abdominal segment. 8, eighth abdominal segment. 9, ninth abdominal segment.

seventh pair of abdominal spiracles are slightly larger than those of the first six abdominal segments. The eighth pair are heavily chitinised and are as large as those of the prothorax.

Chaetotaxy (Text-fig. 5). In the following descriptions Fracker's⁽⁸⁾ method of designating the setae has been adopted. The cephalic plate bears the normal number of six setae on either side above the spiracle; α, γ and ε in an anterior row, and β, δ and ρ in a posterior row. On the

prothorax, the K-group consisting of κ and n are present in a position immediately anterior to the spiracle, and below these, just above the prothoracic leg, are π and ν of the Π group. τ is absent but σ is present, although visible only under high magnification. Fracker⁽⁸⁾, accepting Dyar's⁽²⁾ setal map of *Hepialus*, points out the absence of σ on the prothorax of the first stage of this larva, and states that this seta is also absent in the first stage larva of *Feltia gladaria*. The writers do not know whether the presence of σ on the prothorax of the first stage larva is constant for all Micro-lepidoptera, but Fisher⁽⁷⁾ states that it is present on the first instar larva of *Tortrix pronuba* Hub.

The arrangement of the setae on the mesothorax is identical with that on the metathorax. κ is present in a position just above the level of the spiracle; above κ are α and β , placed together near the median dorsal line of the larva. ϵ and ρ are similarly grouped, on a line parallel to the margin of the segment but in a more lateral position. π is present a short distance above the corresponding thoracic leg, while δ is an absentee.

The setal maps of segments 1 to 8 of the abdomen are very similar. On all the segments α is more anterior and nearer the median dorsal line than β ; λ , δ and ϵ are absent. ρ is present above the spiracle, in all except segment 8, where it is anterior to, and level with the spiracle. The position of κ and n on these segments is typical of the Micro-lepidoptera. κ has migrated into a position anterior and dorsal to π and both are present on the same tubercle. ν and π occur together below the K group, and on segments 3 to 6 are immediately above the prolegs. ρ is present in its normal position on all segments. In segment 9 α is ventral to β , otherwise the arrangement of the setae is similar to that of the other abdominal segments.

The infra-anal comb. An *infra-anal comb* is present beneath the anus. It consists of a chitinous flap drawn out into six spines. These comprise two long median spines, two slightly shorter spines, one on each side of these, and two very short spines one on each outer margin.

The thoracic legs. The thoracic legs (Text-fig. 6) are lightly sclerotised and taper from the coxa to the single-jointed tarsus, which ends in a minute claw.

On its inner surface the *coxa* (*c.*) bears three bristles of equal length, and on its posterior margin one long and one extremely short bristle.

The *trochanter* (*t.*) is extremely short and is devoid of bristles.

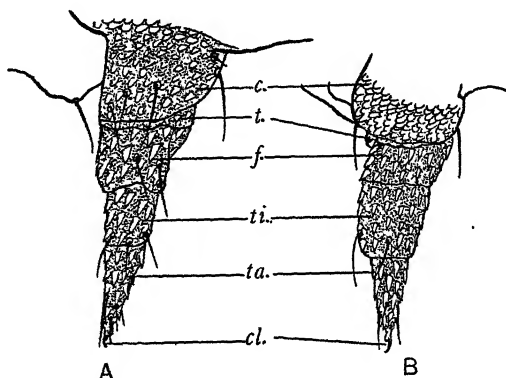
The *femur* (*f.*) has only two bristles which are borne on its inner surface.

The *tibia* (*ti.*) bears at the apex, on its inner surface, three bristles of equal length and on the outer surface one shorter bristle.

At the apex of the *tarsus* (*ta.*) on the inner surface there are two bristles, one long thick bristle immediately posterior to the *claw* (*cl.*), and one short finer bristle a little more removed. On the anterior surface immediately above the claw is a single thick bristle of medium length, and a short fine bristle is present near the apex on the outer surface.

The structure of the thoracic legs is similar in all instars, except that the amount of chitinisation increases with the stages.

The prolegs. Each of the abdominal prolegs is furnished with a complete circle of 9 or 10 crotchets (Text-fig. 7, 1) in uniordinal series.



Text-fig. 6. Thoracic leg of first stage larva ($\times 205$). A, right metathoracic leg (inside).

B, right metathoracic leg (outside). c. = coxa; cl. = claw; f. = femur; t. = trochanter; ta. = tarsus; ti. = tibia.

The same number of crotchets occur on the anal prolegs, but the circle is incomplete.

Biology. The first stage larvae are extremely active and begin to crawl rapidly over the lower branches of the tree almost immediately after hatching. On reaching the leaves they begin to feed, mainly on the under-surface. Some of them spin a few threads of silk around themselves, others suspend themselves in mid-air on a strand of silk or let themselves down to the ground. This wandering habit of first stage larvae was also observed in *C. rosaciana* by Sanders and Dunstan (19), who state that owing to the consequent heavy mortality this is an important factor in their control.

In the laboratory larvae were easily reared on willow. The average duration of this instar was $7\frac{1}{2}$ days, varying from 6 to 10 days. In

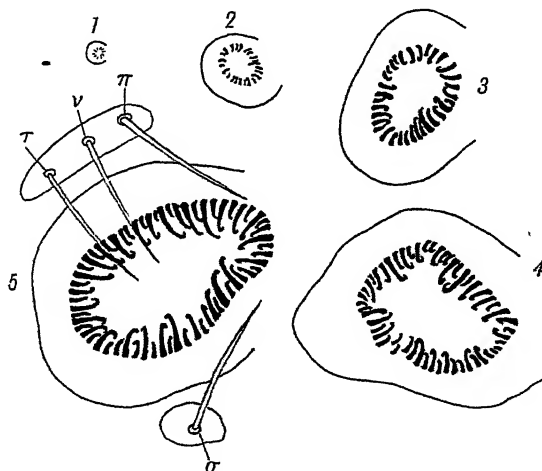
the field during 1931, first stage larvae were present on the trees from April 15th to May 20th.

(2) *Second instar.*

In this stage the body is about 6.5 mm. long and the head capsule 0.45 mm. in breadth, varying between 0.41 and 0.51 mm.

The differences between the first stage larval mouth-parts and those of this and succeeding stages will be described later.

The anal plate is now much larger and more distinctly marked. The areas surrounding the body setae are also larger, blacker, and more heavily chitinised; while the whole body is darker than in the preceding instar.



Text-fig. 7. 1 to 5, crotchets of corresponding instars.

Chaetotaxy (Text-fig. 8). The setal plan of the second stage larva differs from that of the first, in the appearance on the thoracic segments, of the seta θ , in the presence of the seta μ on all the abdominal segments, and of τ on abdominal segments 1 to 8.

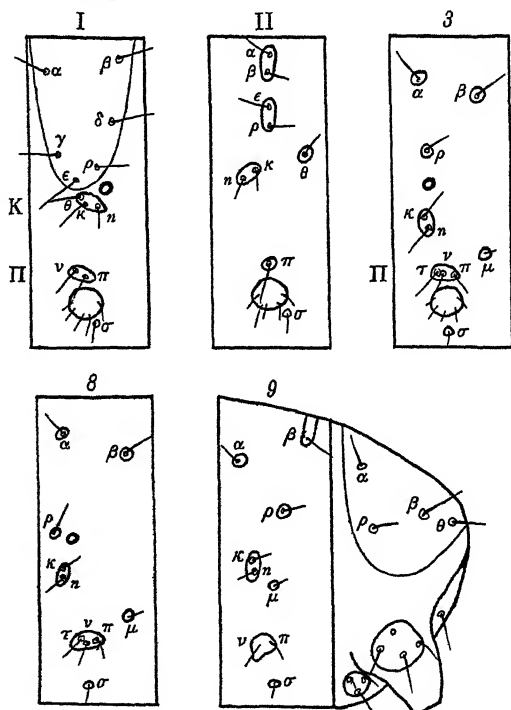
On the prothoracic segment θ has migrated into the K group, but on the meso- and meta-thoracic segments, θ is in its normal position posterior and dorsal to this group.

On the abdominal segments 1 to 8, τ is the anterior seta of the Π group, while μ is present on all the abdominal segments, near the posterior margin and just above the level of the Π group.

In a single larva there was an abnormal seta present below the level of the spiracle on the eighth abdominal segment; and in one instance, on

the eighth abdominal segment, μ , instead of being present in its normal position was included in the K group.

The anal plate being more extensive, now includes the setae β and π , and the setal group on the prolegs includes a puncture which was not present in the first stage larva.



Text-fig. 8. Setal map of second instar larva.

The infra-anal comb. This has seven spines, one very short one on either margin and two pairs of longer spines on either side of a still longer median spine.

The prolegs. The number of crotchets (Text-fig. 7, 2) on the prolegs has increased to 22 or 23; they are still uniordinal, but very irregular in size, some being twice as long as others.

Biology. In this instar the larva is much less active; it continues to feed on the under-surface of the leaf under the cover of a fine web.

The average duration of this instar in the laboratory was $6\frac{1}{2}$ days, with a variation of 5–9 days. In the field second stage larvae were to be found from May 8th to June 3rd.

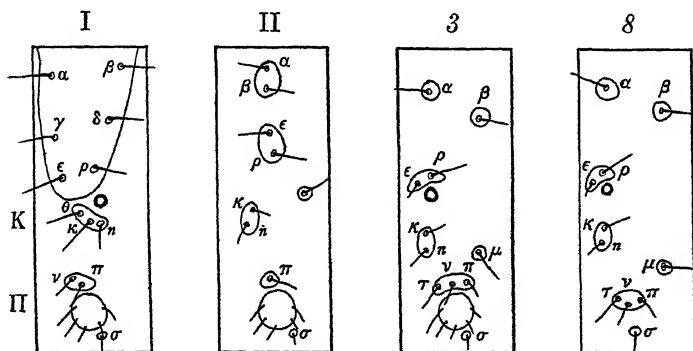
(3) *Third instar.*

The total length is 13 mm. It is much darker than in the preceding stages, being a dull black. Occasionally, however, a third instar larva has been observed to be almost as pale as a second stage.

The head capsule is 0.73 mm. across, it may vary from 0.65 to 0.76 mm.

Chaetotaxy (Text-fig. 9). The addition of the seta ϵ to the abdominal segments numbers 1 to 8 is the only feature which distinguishes the setal map of this instar from that of the preceding stage.

The infra-anal comb. The *infra-anal comb* is also similar and differs only in that the marginal spines are comparatively longer.



Text-fig. 9. Setal map of third instar larva.

The prolegs. The crotchets (Text-fig. 7, 3) are still present in uniordinal series, though there is a large amount of variation in their lengths. The number of crotchets per series is now 32 to 35, with incomplete circles on the anal prolegs, as in the previous instars.

Biology. The third stage larva feeds inside the rolled edges of the leaves. It fastens strands of silk from the edges of the leaves to the under-surfaces; these strands shorten on drying, so that the leaf edges are pulled down until they eventually meet the lower surface, thus completely enclosing the larva.

When feeding on willow in the laboratory the leaves were so narrow that both edges were drawn together to form a tube.

This instar has an average duration of $6\frac{1}{2}$ days; it may vary from 5 to 8 days. Third stage larvae were also present in the field from May 18th to June 3rd.

(4) *Fourth instar.*

The larva is darker and measures about 18 mm. in length; the head capsule is 1.23 mm. wide, varying from 1.08 to 1.33 mm.

Chaetotaxy. The setal map is identical with that of the preceding instar.

The infra-anal comb. This has now eight spines; six shorter spines are arranged symmetrically in order of length, on either side of a pair of long median spines.

The prolegs. The crotchets (Text-fig. 7, 4) are now almost in biordinal series and are 52 to 56 in number. The series is not perfectly regular, as in some parts of the circle three or four short crotchets may occur together.

Biology. The larva, now being much larger, feeds voraciously and often eats its way through the sides of the rolled leaves. When this occurs, strands of silk are fastened to other leaves, which are thus pulled down to enclose the larva in a complete canopy.

The duration of this instar in the laboratory was 6 days. In the field larvae were found from June 3rd to June 18th.

(5) *Fifth instar.*

This mature larva attains a length of 23 mm., and is the same dull black as the preceding stage.

The head. The *head capsule* is shining black as in all the other stages, and its *setal map* is also identical with those of the third and fourth instar larvae.

The *labrum* (Text-fig. 10 B) is similar to that of the first stage larva.

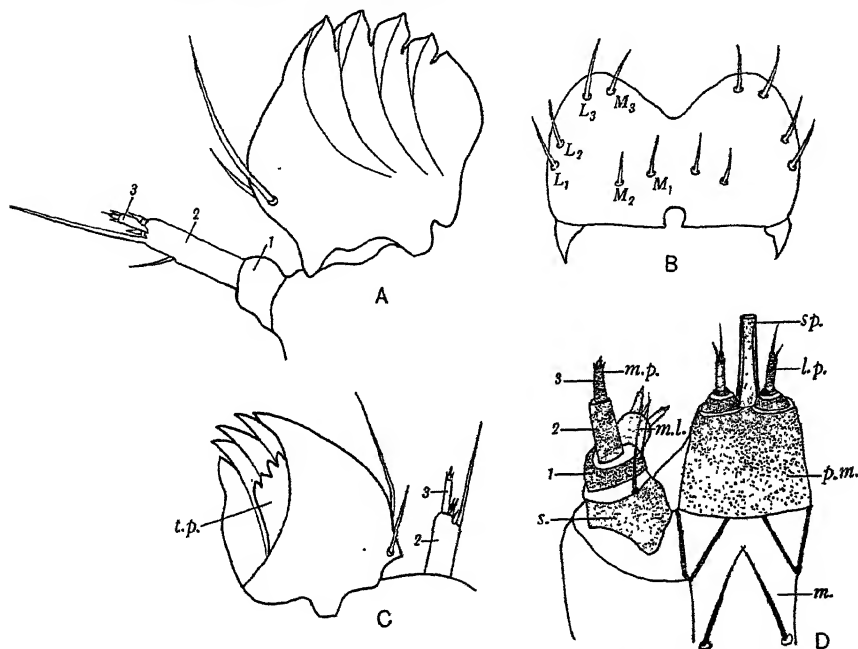
The *antennae* (Text-fig. 10 A) throughout all the preceding stages have the second joint gradually elongated, until it is now three times the length of the first. The second joint bears at its apex, one on each side of the base of the third joint, two small, two-jointed, cone-like papillae, and immediately adjacent to the outer cone a long seta. There is a short seta on the lateral margin about one-third of the distance from the apex to the base. The third joint is surmounted by a papilla which terminates in a short fine bristle, and two cone-like papillae one on each side of the terminal papilla.

The mouth-parts. The *mandibles* (Text-fig. 10 A) in shape and setal arrangement are similar to those of the first stage larva. They are, however, wider in proportion to their length, the outside edges having an almost semicircular outline.

Each mandible has on its ventral surface a flat chitinous plate which

bears four short teeth. This plate is present in all the instars except the first.

The *maxillae* (Text-fig. 10 D) are essentially similar to those of the first stage larva; the main difference being the arrangement of the papillae on the *inner lobe (m.l.)*. This lobe bears at its apex two stout two-jointed cone-like papillae; and on its dorsal surface near the apex two more, long, single-jointed papillae.



Text-fig. 10. Mouth-parts of fourth and fifth instar larvae ($\times 66$). A, left mandible and antenna of fifth instar larva (dorsal). B, labrum of fifth instar larva (dorsal). C, left mandible and antenna of fourth instar larva (ventral). D, maxilla and labium of fifth instar larva (ventral). *l.p.*=labial palp; *m.*=mentum; *m.l.*=maxillary lobe; *m.p.*=maxillary palp; *p.m.*=prementum; *s.*=stipes; *sp.*=spinneret; *t.p.*=toothed plate; *L* and *M*=setae.

The *labium* (Text-fig. 10 D) is comparatively shorter and more chitinated than in the preceding stages. Each labial palp bears at its apex a short papilla which ends in a fine bristle, and immediately adjacent to this on the outer margin is a shorter stiff bristle. The spinneret at this stage assumes the form of a hollow cylinder which is devoid of papillae.

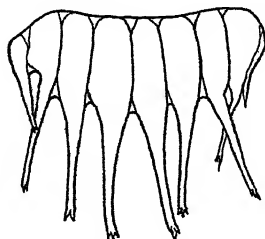
The *spiracles* (Text-fig. 4). These are similar to those of the first stage larva and are the same in the intermediate stages.

Chaetotaxy. The setal map is identical with that of the third and fourth instar larvae.

The infra-anal comb. The *infra-anal comb* (Text-fig. 11) is similar to that of the fourth stage larva, but an additional small marginal spine may be present. The spines at their apices may be produced into two or three short barbs.

The prolegs. The crotchets (Text-fig. 7, 5) may now be regarded as a biordinal series, though there is still a certain amount of irregularity; but not more than two short crotchets occur together. The number of crotchets per series varies from 61 to 68.

Biology. The habits of the fifth stage larvae are similar to those of the fourth. In this stage the larvae are to be found in the rolled up leaves generally at the ends of the shoots.



Text-fig. 11. Infra-anal comb of fifth instar larva.

The duration of this instar in the laboratory varied from 9 to 10 days and averaged $9\frac{1}{2}$ days. In the field larvae were found from June 19th to June 29th.

(d) *The larva in relation to Dyar's hypothesis.*

Dyar(2) has stated that "the widths of a head of a larva in its successive stages follow a regular geometrical progression." This hypothesis has been found to be true for the larva of *C. crataegana* (Plate XXVII, fig. 7). Table I gives in millimetres the widths of the unmounted head capsules of the successive stages and the calculated widths, taking 1.64 as the factor.

Table I.

Head measurements of larvae of C. crataegana in successive instars.

Stage	Measurements of larval heads in mm.	Calculated widths in mm.
1st	0.274	—
2nd	0.45	0.45
3rd	0.73	0.74
4th	1.23	1.21
5th	1.93	1.98

(e) *The pupa.*

A brief description of the pupa (Plate XXVII, fig. 8) has been given by Barrett⁽¹⁾ and by Sheldon⁽²⁰⁾. It is dull black, with shiny black wings. The male has an average length of 9.6 mm. and the female of 12.3 mm.

The head and thorax are typical of a Tortricid pupa; the wing cases and antennae are sealed down to the body and reach the second abdominal segment. In the male, abdominal segments 4, 5, 6 and 7 are moveable, and in the female the same segments with the exception of the seventh are also free.

In addition to the setae on the abdominal segments, which are present in positions homologous with those on the mature larva, there are two short rows of spines (Text-fig. 12) on the dorsal surface of abdominal segments 2 and 8; one row near the anterior and one near the posterior margin.

The abdomen terminates in a fairly long, drawn-out, heavily chitinated *cremaster* (Text-fig. 13). This bears at its apex four stout hooks (*h.*), and on the dorsal surface two pairs of hooks arise near the lateral margins; one pair slightly removed from the apex and another pair immediately behind these.

The spiracles. There are seven pairs of abdominal spiracles; the first three pairs being hidden beneath the pupal wings. There is also a rudimentary pair on abdominal segment 8.

Male genitalia (Text-fig. 12). The male *genital aperture* (*g.a.*) is present on the ventral surface of the ninth abdominal segment. It is a short narrow depression lying between two raised labia. The *anus* (*a.*) is long and slit-like and situated on the ventral surface of the tenth segment.

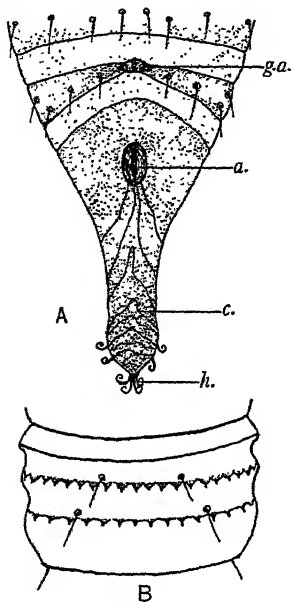
Female genitalia (Text-fig. 13). In the female there are two *genital apertures*, the opening of the *bursa copulatrix* (*b.c.*) and the external orifice of the oviduct (*o.a.*).

On its ventral surface the eighth abdominal segment is narrowed towards the median line. The ninth is much narrower and each side is extended forward to form an apex, which almost projects onto the posterior margin of the preceding segment. The intersegmental lines of these two segments do not meet on the ventral surface. The opening of the *bursa copulatrix* is situated in the median line between the apices of the two sides of the eighth segment, and the oviduct opens immediately posterior to this, in a similar position on the ninth abdominal segment. A slit-like *anus* (*a.*) is present on the ventral surface of the tenth abdominal segment.

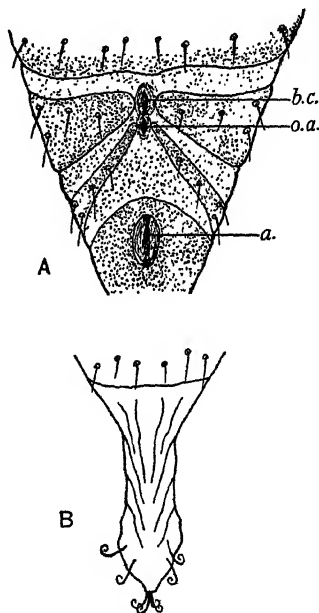
Biology. The fifth stage larva generally pupates inside a rolled leaf, or between two leaves spun together. In some cases, however, pupae

have been observed hanging suspended from a leaf or twig by the hooks on the cremaster (Plate XXVII, fig. 8). The empty pupa cases generally project some distance from the leaves which formerly protected them.

In the laboratory, in a very few instances, larvae pupated after the fourth stage.



Text-fig. 12.



Text-fig. 13.

Text-fig. 12. A, ventral view of posterior end of male pupa ($\times 27\frac{1}{2}$). a.=anus; c.=cremaster; g.a.=genital aperture; h.=hooks. B, sixth abdominal segment of pupa (dorsal) ($\times 14$).

Text-fig. 13. A, ventral view of posterior end of female pupa ($\times 27\frac{1}{2}$). a.=anus; b.c.=bursa copulatrix; o.a.=aperture of oviduct. B, dorsal view of cremaster of female pupa ($\times 27\frac{1}{2}$).

The duration of the pupal instar was found to vary from 10 to 31 days. The average duration noted in the laboratory was 20 days.

In the field, pupae were found from June 1st to July 9th.

(f) *The moth.*

In addition to being figured by Wood⁽²⁵⁾ and Morris⁽¹⁷⁾ the moth is very well described by Barrett⁽¹⁾, so that no description is attempted here.

Biology. The habits of the moth are also described by Barrett⁽¹⁾, and several writers record it as on the wing in July. In the laboratory the

moths remained hidden amongst the leaves during the day, but at night they were very active, darting rapidly about the cage. They also fed during the night, and seemed to show a preference for wild Umbelliferae and wallflowers.

In the field, a pupa from which the moth had emerged was found on June 19th, and on June 23rd several moths hatched in the laboratory from pupae brought in on June 19th.

In the laboratory moths were observed copulating within four days of emergence, but over a week elapsed before egg-laying took place.

III. ECONOMIC IMPORTANCE.

(a) *Host plants and distribution.*

The host plants of *C. crataegana* in Great Britain, recorded in the introduction, are fruit trees, aspen, poplar, oak, hazel, birch, sycamore and sallow. The moth is recorded as being found in woods and of somewhat local occurrence.

In the Wisbech area the egg-masses were found in only three localities; these were all within a radius of seven miles of Wisbech. The first was in a large orchard at Leverington, on apple, plum, and pear; the second at Tilney St Lawrence, near King's Lynn, on plum and apple; and the third at Terrington St John, near Wisbech, on cherry. In the last instance, although apple trees were present adjacent to the cherries, only very few egg-masses could be found on them. Careful search on other recorded food plants near these three centres entirely failed to reveal the presence of any egg-masses.

(b) *Injury to plants.*

Since the larva of *C. crataegana* feeds only on well-developed leaves, it is not of very great economic importance in commercial orchards, unless present in large numbers.

IV. CONTROL.

No attempt was made to devise means of control, but observations were made on the effect of ordinary routine spraying on the egg-masses and larvae.

The trees on which the pest was found were winter sprayed with various types of tar oil and mineral oil washes. Ordinary tar oil washes applied at strengths varying from 5 to 10 per cent., Long Ashton two solution washes at 10 per cent., modified Long Ashton washes at 10-12 per cent., and mineral oil emulsions at 7½-10 per cent. In one instance

the apple trees were sprayed with an ordinary tar oil at $7\frac{1}{2}$ per cent., followed by a mineral oil emulsion at $7\frac{1}{2}$ per cent.

Egg-masses from treated and untreated trees were kept in the laboratory, and it was found that the percentage of larvae hatching was the same in all cases.

Routine spraying with lead arsenate appeared to have very little effect upon the larvae, owing to their habit of feeding inside the rolled up leaves where the spray could not penetrate.

The wandering habit of the first stage larva is probably a factor in checking the increase of this pest. This, together with the effects of parasitism, has probably prevented it from causing serious damage.

In one orchard, where there are several rows of apple trees adjacent to cherry trees infested with *C. crataegana*, the owner has noticed that in their early stages the larvae are frequently blown off the tall cherry trees onto the nearby apples, many of them becoming lost in the loose soil and vegetation. Although some of the larvae complete their life cycle upon the apples, the moths appear to return to the cherries to oviposit, because egg-masses are rarely found upon the apple trees.

V. PARASITES.

There does not appear to be any record of parasites of *C. crataegana*.

From larvae collected in the field one Tachinid and four Hymenopterous parasites were obtained.

On June 19th a larva of the ectoparasitic Ichneumon *Phytodietus segmentator* Grav. (Plate XXVII, fig. 9) was found, firmly attached by means of its pincer-like jaws to the second thoracic segment of a fifth stage larva of *C. crataegana*. Two days later this larva was dead; the parasite which was now attached to the last abdominal segment of the caterpillar had moulted and was about 0.75 mm. long, cream in colour, with a pink coloration beneath the skin. The cast skin of the parasite remained attached to the second thoracic segment of the caterpillar. If removed from its host the parasite quickly seized the dead body and buried its jaws deeply into the flesh. On June 22nd it spun a very rough, brownish, silk cocoon and started to pupate. The adult parasite emerged on July 13th.

On July 7th two females of the Ichneumon, *Pimpla maculator* F., were bred from pupae of the moth.

From a parasitised caterpillar isolated on June 25th, a number of larvae of the Braconid, *Macrocentrus abdominalis* F., emerged. On pupation the individual cocoons were bound together by strands of silk

(Plate XXVII, fig. 10). On July 12th and 13th these gave rise to three males and twenty-one females.

One female of another Braconid, *Apanteles xanthostigmus* Hal., emerged on July 7th.

A Tachinid, *Exorista (Zenillia) roseanae* B. & B., emerged on July 7th, from a Dipterous pupa found on June 23rd amongst pupae of the moth.

In addition to the larval parasites, an egg parasite, *Trichogramma evanescens* Westw., emerged on January 20th, 1930, from egg-masses of *C. crataegana* which had been kept in the laboratory for some time.

In 1931 the writers determined to investigate more fully the life history of this parasite.

Marchal⁽¹²⁾ has shown that this Chalcid may be of two types, one, *T. evanescens*, the type species, is distinguished by its dark coloration and its iridescent wings which have a blue tint. This may have as many as eight generations a year and parasitises Lepidopterous larvae, especially caterpillars of the cabbage butterflies, it also parasitises Syrphid eggs. The other type Marchal designates *T. cacoeciae*; this is distinguished by its clear coloration and its iridescent wings which have a rose tint. It has only two generations a year and parasitises the eggs of *C. rosana*, which are to be found on the bark of quince and apple trees.

Thorpe⁽²²⁾ regards the two types as two well-differentiated biological races.

The eight generations of *T. evanescens* consist of both males and females. Marchal isolated the females which then reproduced parthenogenetically to give rise to males.

T. cacoeciae has two dissimilar generations which generally consist of females only. The summer generation in June and July is normally winged, whereas the spring generation has vestigial wings.

In the laboratory, several parasites hatched from egg-masses of *C. crataegana* on March 24th and 25th. The holes they made in the egg-mass on hatching were circular (Plate XXVII, fig. 11), and were easily distinguished from the elliptical holes made by the caterpillars. On March 26th, two days after the first parasites hatched, the larvae of *C. crataegana* began to emerge. The parasites on hatching were isolated, each with a fresh egg-mass; within 24 hours several of them were observed to be ovipositing. The egg-masses in which the parasites had oviposited were carefully watched, and on April 28th, after the elapse of just over one month, a second generation of parasites appeared. Unfortunately the egg-masses of *C. crataegana* in the field had all hatched, and no other eggs being available further observations could not be made.

Another lot of parasites hatched and oviposited on April 15th; from these a second generation emerged on May 20th, just over a month elapsing between the two generations as in the previous instance.

The number of parasites hatching from one egg-mass varied from 1 to 9; the average number was 3. About 5 per cent. of the whole of the egg-masses were parasitised.

All the members of both generations were females with perfect iridescent wings having a blue sheen, and therefore, according to Marchal, must be *T. evanescens*. These were all females, whereas those he bred out were of both sexes.

According to Marchal the summer generation of *T. cacoeciae* lays its eggs in the middle of the egg-masses of *C. rosana*, the periphery is left untouched. These hatch the following March or early April and constitute the vestigial-winged generation. Without moving away, these oviposit in the periphery of the egg-mass, and from these eggs the fully winged summer generation emerges in July.

In the field *T. evanescens* had emerged on April 13th, two days before the caterpillars hatched. The writers failed to find the second generation in the field, but if each generation requires just over a month to complete its life cycle as in the laboratory, then at the most there can only be four or perhaps five generations a year.

The most interesting point about the life history of this parasite is that it oviposits a short time before the caterpillars are due to hatch from the egg-masses and does not confine its attentions solely to the periphery as does *T. cacoeciae*, but instead ranges over almost the whole surface. Furthermore the first generation hatch from both the periphery and the middle of the egg-mass. Wherever the ovipositor of the parasite was inserted, no caterpillar hatched from that egg. After the parasites had hatched a dissection was attempted to determine whether any head capsules of the caterpillar remained, but none could be found.

The writers hope to clear up this point another year, and to obtain a third and perhaps more generations of the parasite and so determine if this particular type can be regarded as a distinct biological race.

The act of oviposition was watched several times in the laboratory. The parasite runs quickly over the surface of the egg-mass tapping both antennae alternately at an enormous speed. Suddenly she decides on a certain spot and turns round so that her abdomen is above the spot she has chosen, and then draws out her ovipositor and drives it into the egg-mass. Sometimes a female may lay one egg, then test the surface again with her antennae, move on a few paces and lay another. One

female was watched for five hours, and during that time she laid five eggs; another female laid three egg in three hours.

VI. SUMMARY.

An account has been given of the life history and habits of the moth *Cacoecia crataegana*, in the Wisbech area, where it attacks fruit trees.

The egg and egg-laying habits have been described and the external anatomy and biology of the larva and pupa have been studied.

The measurement of the larval head capsule in relation to Dyar's hypothesis has been discussed.

The effect on the egg and on the larva of routine control measures has been observed.

The parasitism of the moth has been studied. One Dipterous, and five Hymenopterous parasites have been bred out, and the biology of one of these, the egg parasite *Trichogramma evanescens* Westw., has been studied in detail.

The writers wish to acknowledge their indebtedness to Mr F. R. Petherbridge, under whose supervision the work has been carried out, and to Dr A. D. Imms and Mr J. C. F. Fryer for criticism of the manuscript. They also wish to thank members of the staff of the British Museum of Natural History, for identification of the moth and its parasites, and for supplying references to the literature, and to Mr M. H. Hey, of the Mineralogical Department, for the analysis of the egg-masses. Thanks are also due to Messrs J. Lefevre, E. G. Davison, and G. E. Munday, for reporting the presence of egg-masses at the various centres, and to the growers in whose orchards the observations were made.

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EXPLANATION OF PLATES XXVI AND XXVII.

PLATE XXVI.

- Fig. 1. Unhatched egg-mass of *C. crataegana* ($\times 5$).
- Fig. 2. Ventral view of egg-mass to show honey-comb formation ($\times 16.6$).
- Fig. 3. Transverse section of egg-mass ($\times 13$).
- Fig. 4. Egg-masses of *C. crataegana* on plum tree.
- Fig. 5. Transverse section through centre of egg-mass to show brick-like formation ($\times 25$).
- Fig. 6. Hatched egg-masses ($\times 5$).

PLATE XXVII.

- Fig. 7. Larval head capsules to show the comparative sizes in the five stages ($\times 6.5$).
- Fig. 8. The pupa ($\times 3$).
- Fig. 9. Larva of *Phytodietus segmentator* Grav. attached to thorax of fifth stage larva of *C. crataegana* ($\times 3.6$).
- Fig. 10. Cocoons of *Macrocentrus abdominalis* F. ($\times 1.4$).
- Fig. 11. Egg-masses of *C. crataegana* showing holes made by *Trichogramma evanescens* Westw. ($\times 6$).

(Received October 25th, 1932.)



Fig. 1.



Fig. 2.



Fig. 3.



Fig. 4.



Fig. 5.



Fig. 6.



Fig. 7.



Fig. 9.



Fig. 8.



Fig. 10.



Fig. 11.

OBSERVATIONS ON THE DESERT LOCUST IN EAST AFRICA FROM JULY, 1928 TO APRIL, 1929

BY C. B. WILLIAMS, M.A., Sc.D.

(Formerly Entomologist to the East African Agricultural Research Station,
Amani, Tanganyika Territory. Chief Entomologist, Rothamsted
Experimental Station.)

(With Plate XXVIII and 10 Text-figures.)

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INTRODUCTORY.

EARLY in 1928 word came through to the newly reopened Agricultural Research Station at Amani, Tanganyika, that swarms of locusts had appeared in various parts of Kenya Colony, after an absence of many years. Very few of these swarms reached as far south as Tanganyika but egg-laying was reported to have taken place in several localities in Kenya. The species was *Schistocerca gregaria* Forsk.

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In July, 1928, it was suggested that the Entomologist at Amani should visit Kenya and investigate the locusts, particularly from the biological side, leaving problems of immediate control measures to the local Department of Agriculture and their Entomologists. As a result I visited the Rift Valley in Kenya in July and August, 1928, and was able to make observations at Naivasha and Gilgil on swarms of medium and large hoppers and just emerging adults. Practically no hoppers smaller than stage III were seen. I then returned to Amani carrying with me a number of last stage hoppers and adults in the hope that I would be able to breed them in captivity and to make some experiments on the influence of crowding and isolation on the production of the gregarious and solitary phases. This attempt failed as all the specimens I took back died within three weeks, largely I believe owing to the unusually cool and damp weather that followed.

In November and December, 1928, I again visited Kenya, and made observations on swarms of yellow, mature adults, on their oviposition and on a few swarms of just hatched hoppers. Mature adults were also seen in the Moshi district of Tanganyika.

I returned to Amani in the middle of December, with a large number of first stage hoppers, and was able to make a few experiments on the effects of isolation. The somewhat inconclusive results are given below.

Up to this time no locusts in any stage had appeared in the Amani district. On January 29th, 1929, however, a very large swarm of immature, purple-brown adults, invaded this area. From that date till the beginning of April, when I left East Africa, swarms were constantly observed in the district, but no mature adults were seen and no egg-laying took place.

The observations here recorded were at the time considered merely as a preliminary survey for a more complete investigation later. This, however, never materialised as I did not return to East Africa.

According to Morstatt⁽¹⁾ locusts had previously appeared in East Africa in 1893, 1898, 1903-5 and 1913-14. The 1903-5 swarm is fully described by Vosseler⁽²⁾. The 1913-14 outbreak was small, and only one specimen for this year was in the collections at Amani. In February, 1914, locusts were said to be breeding in the districts of Irangi and Dodoma in Tanganyika according to Lounsbury⁽³⁾.

The intervals between the 1903-5 outbreak, the 1913-14 outbreak and the present one (1927) are therefore 10 and 14 years, but the two earlier swarms (assuming them to be the same species) were much closer together.

FIELD OBSERVATIONS.

General notes on life cycle.

No attempt was made to work out the normal details of the life cycle, but in the course of work the following points were noted which perhaps may be useful to other observers.

From eggs laid in the Rift Valley apparently in April or May, 1928, hoppers hatched and reached the adult stage at the end of July and in August, 1928. The flyers remained in the purple-brown stage for some months, but about the end of October reports of yellow locusts first came to hand, and towards the end of November egg-laying was reported. Egg-laying was general in November and December, and by December 11th, and probably earlier, eggs had already begun to hatch. In captivity at Amani hoppers from these eggs bred slowly, but at the end of January swarms of purple brown adults invaded North-east Tanganyika which were probably from eggs laid early in November. Thus two broods were passed through in about one year.

At Bura, Kenya, in December, 1928, a large number of egg-masses were dug up in the search for parasites. The number of eggs in each of 36 holes varied from 14 to 80 and averaged 46.

Pairing and egg-laying was extensive in the Bura district on December 6th-10th, 1928. The male remained on the back of the female during egg-laying (Plate XXVIII, fig. 2). Egg-laying continued till well after dark. Pairing took place before egg-laying, but I was not able to settle whether it happened again after egg-laying.

The holes bored for egg-laying were about $3\frac{1}{2}$ in. to the extreme bottom, the egg-mass at the bottom occupying about $1\frac{1}{2}$ in. and the froth-filled tunnel about 2 in. The top $\frac{1}{4}$ in. or so of the tunnel was filled with earth so that the holes were not conspicuous from above, but by cutting away with a large knife the top $\frac{1}{2}$ in. of the soil the froth in the holes appeared as round white patches. This was a rapid method of estimating the number of egg-masses in any given area.

Sex proportion.

The following observations on the sex proportion in adult swarms were recorded. Females preponderate slightly in four out of the five observations.

Date	Locality	Stage	Males	Females
1. viii. 28	Naivasha, Kenya	Purple-brown adults	11	15
18. ii. 29	Amani	"	61	78
18. ii. 29	"	"	59	56
18. ii. 29	"	"	117	130
19. ii. 29	Bulwa, near Amani	"	48	58
Total			296	337

466 *Observations on the Desert Locust in East Africa*

Food of adults.

Observations made at Amani, Tanganyika.

Not eaten or only very slightly eaten

Regularly eaten

ACANTHACEAE.

Sanchezia parvebractea Sprague and Hutch.
Strobilanthes Dyerianus Mast.

AMARANTACEAE.

Gomphrena globosa L. (Leaves eaten, flowers left)

AMARYLLIDACEAE.

Furcraea gigantea Vent.

ANACARDIACEAE.

Spondias cytherea Sonnerat.
Mangifera (slight) (Mango)

ANNONACEAE.

Annona muricata L.
Annona glabra L.

APOCYNACEAE.

Conopharyngia Holstii Stapf
Lanugina variegata N. E. Br. (East Africa can rubber)
Nereum Oleander L.
Plumeria acuminata Ait. (Frangipani)
Strophanthus hispidus A. P. DC.

Landolphia spp. (Rubber vines)
Thevetia nereifolia Juss.

AQUIFOLIACEAE.

Ilex paraguayensis A. St Hil. (Paraguay tea)

BIGNONIACEAE.

Jacaranda mimosifolia D. Don (slight) *Tecoma stans* Juss.

BOMBACACEAE.

Durio zibethinus Murr. (Durian) (top of tree only, not touched first flight)

BROMELIACEAE.

Ananas comosus Merr. (pineapple)

CAESALPINIACEAE.

Cassia Fistula L.
Erythrophloeum guineense G. Don
Trachylobium verrucosum Oliv.

Cassia sp.
Schizolobium excelsum Vog.

CANNACEAE.

Canna generalis Bailey

CARICACEAE.

Carica Papaya L. (Pawpaw) (slight at Amani, severely damaged in the plains)

Not eaten or only very slightly eaten

Regularly eaten

CASUARINACEAE.

Casuarina sp. (defoliated first visit)

COMPOSITAE.

Dahlia sp. (very slight)
Notonia amaniensis Engl.
Zinnia sp. (slight)

CRUCIFERAE.

Brassica oleracea L. (cabbage)

CUCURBITACEAE.

Cucurbita Pepo L. (pumpkin)

CYCADACEAE.

Cycas circinalis L.
Encephalartos Hildebrandtii Br. & Bouché

CYCLANTHACEAE.

Carludovica palmata Ruiz. & Pav.
 (Panama hat palm)

DILLENIACEAE.

Dillenia indica L.

EUPHORBIACEAE.

Euphorbia sp.
Jatropha sp.
Ricinus communis L. (castor oil) (very slight)
Codiaeum variegatum Blume (croton)

Acalypha sp.
Manihot utilissima Phol. (cassava) (very severe)
Manihot Glaziovii Müll. Arg. (Ceara rubber) (slight to distinct)
Phyllanthus sp.

EBENACEAE.

Diospyros sp.

GRAMINEAE.

Panicum maximum Jacq. (very slight)
Pennisetum Ruppelii Steud.
Pennisetum purpureum Schum. (elephant grass)
Shibataea kumasasa Makino

Chimonobambusa Hookeriana Nakai (much liked)
Dendrocalamus strictus Nees
Zea Mays L. (maize)

GUTTIFERAE.

Allanblackia Stuhlmannii Engl.
Garcinia ferrea Pierre

HYPERICACEAE.

Harungana madagascariensis Lam.

LABIATAE.

Mentha viridis L. (mint)
Origanum vulgare L. (marjoram)
Salvia splendens Ker

LAURACEAE.

Laurus nobilis L. (bay)

Cinnamomum zeylanicum Breyn. (tops of trees only)
Persea americana Mill. (Avocado pear) (tops of trees damaged on third visit)

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Not eaten or only very slightly eaten

Regularly eaten

LECYTHIDACEAE.

Couroupita guianensis Aubl. (slight)

LILIACEAE.

Asparagus plumosus Baker

Dasyllirion glaucophyllum Hook.

Pleomele Papahu N. E. Br.

Yucca aloifolia L.

LOGANIACEAE.

Anthocleista orientalis Gilg (slight)

LORANTHACEAE.

Loranthus sp.

MAGNOLIACEAE.

Michelia Champaca L.

MALVACEAE.

Hibiscus schizopetalus Hook. f.

Hibiscus esculentus L. (Okra)

MELIACEAE.

Cedrela sp.

Sandoricum Koetjape Merr.

Bersama usambarica Gürke

MELIANTHACEAE.

MIMOSACEAE.

Albizia Sassa Macbride

Albizia stipulata Boiv.

Acacia Farnesiana Willd.

MORACEAE.

Artocarpus integra Merr. (slight)

Castilloa elastica Cerv.

Chlorophora excelsa Benth. & Hook, f.

Ficus retusa L.

Treculia africana Decne

Ficus elastica Roxb.

Ficus altissima Blume

Morus nigra L. (mulberry) (even bark eaten)

MUSACEAE.

Musa spp. (banana)

Musa textilis Née (Manila hemp)

MYRTACEAE.

Eugenia Jambos L.

Eugenia malaccensis L.

Eugenia Jambolana Lam.

Eugenia uniflora L.

Eucalyptus sp.

Melaleuca Leucadendra L.

Psidium Catleianum Sabine

Syncarpia laurifolia Ten.

Syzygium guineense DC.

Psidium Guajava L. (slight)

NYCTAGINACEAE.

Bougainvillea (slight on younger shoots)

OLEACEAE.

Jasminum sp.

Not eaten or only very slightly eaten

Regularly eaten

OXALIDACEAE.

Averrhoa Carambola L.

PALMAE.

Cocos eriospatha Mart. ex Drude*Cocos nucifera* L. (coco-nut)*Livistona australis* Mart.*Livistona chinensis* R. Br.*Phoenix humilis* Royle*Trachycarpus excelsa* Wendl.

PANDANACEAE.

Pandanus spp.*Pandanus utilis* Bory

PAPILIONACEAE.

Myroxylon balsamum Harms (very slight)*Cajanus Cajan* Millsp. (pigeon pea)*Pterocarpus Marsupium* Roxb.*Erythrina micropteryx* Poepp. (much eaten)*Pisum sativum* L. (green pea)

PINACEAE.

Cryptomeria japonica D. Don*Araucaria Bidwillii* Hook. (distinct)*Cupressus Goveniana* Gord.*Araucaria Cookii* R. Br. (third visit only)

PIPERACEAE.

Piper angustifolium R. & P.

PLUMBAGINACEAE.

Plumbago capensis Thunb.

PROTEACEAE.

Grevillea robusta A. Cunn (some trees much eaten)

RHAMNACEAE.

Hovenia dulcis Thunb. (much eaten)

ROSACEAE.

Eriobotrya japonica Lindl. (loquat)*Prunus Persica* S. & Z. (peach) (only slight at first, later defoliated)*Rubus rosaeifolius* Sm.

RUBIACEAE.

Cinchona spp. (quinine) (very slight)*Coffea robusta* L. Linden (coffee) (flowers much eaten, not leaves)

RUTACEAE.

Clausena Lansium Skeels*Casimiroa edulis* La Lave*Citrus maxima* Merr. (grape fruit) (tops defoliated)

SAPINDACEAE.

Litchi chinensis Sonn.

SAPOTACEAE.

Chrysophyllum Cainito L.*Mimusops Schimperi* Hochst.

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Not eaten or only very slightly eaten

Regularly eaten

SOLANACEAE.

Cyphomandra betacea Sendt. (tree tomato) *Datura* sp.
Physalis sp.
Solanum sp.
Solanum Lycopersicum L. (tomato)
Solanum Melongena L. (egg plant)

THEACEAE.

Thea sinensis L.

TROPAEOLACEAE.

Tropaeolum majus L. (very slight)

ULMACEAE.

Trema guineensis Ficalho (much eaten)

UMBELLIFERAE.

Daucus Carota L. (carrot)
Petroselinum sativum Hoffm. (parsley)

VERBENACEAE.

Durandtia repens L. (especially flowers)
Lantana Camara L.

FILICALES.

Bracken and all ferns

The interest of this list is chiefly in the very definite likes and dislikes of the insects often with one natural order and sometimes within a single genus of plants.

Of the 62 natural orders mentioned

25 are only represented by edible species.

19 are only represented by inedible species.

18 are represented by both edible and inedible species.

The most obviously sought-after tree was the *Casuarina*, and all of these at Amani were defoliated at the first visit of the locusts. The needle-like leaves were bitten through near the base, and the ground under the trees was rapidly covered with a carpet of green fragments.

Cassava (*Manihot utilissima*) was severely defoliated as also was maize, and these two plants constituted the chief native crops of the district.

Mulberry was severely attacked, even the bark being eaten, but many other members of the order Moraceae were not touched. In the genus *Ficus*, however, two species were damaged and one (*F. nitida*) was untouched.

Both *Erythrina* sp. and *Grevillea* sp., which are largely used for coffee shade trees, were heavily defoliated; but with the coffee itself there was no damage to leaves, although on one occasion all the flowers on several hundred acres were completely destroyed.

The reverse was the case with an ornamental *Gomphrena* growing in one of the gardens; the leaves were completely destroyed while the purple flowers were untouched.

The hard leaves of *Araucaria cookei* were untouched by the first two swarms which visited Amani, but were distinctly damaged by the third swarm which, presumably, found the more attractive foods already eaten.

On the whole the adult locusts showed a distinct, and unfortunate, preference for plants of economic value.

Food of hoppers.

Observations made at Naivasha, Kenya, in August, 1928.

ACANTHACEAE.

Hypoestes verticillaris R. Br., not eaten.

AMARANTACEAE.

Achyranthes asper L., not eaten.

Aerva tomentosa Forsk., not eaten.

AMARYLLIDACEAE.

Agave sisalana Per., slightly eaten.

BORAGINACEAE.

Heliotropium sp., very little eaten.

CHENOPODIACEAE.

Chenopodium opulifolium Sch., inflorescence and red cortex of stem much eaten, leaves only slightly.

COMPOSITAE.

Conyza sp., not eaten.

FICOIDACEAE.

Trianthema pentandra L., only slightly eaten.

GRAMINEAE.

Cynodon plectostachyum (K. Sch.) Pilg., all green parts eaten and dried parts nibbled. One of the standard foods, but very dried up in the Naivasha area at the time of my visit.

Eragrostis sp., much eaten; when growing on roadsides eaten down to ground.

LABIATAE.

Leonotis velutina Fenzl., cortex of stalk only eaten.

Ocimum sp., slightly nibbled.

PAPILIONACEAE.

Crotalaria sp., eaten.

SOLANACEAE.

Solanum indicum L., not eaten.

Datura stramonium L., not eaten.

? *Withania somnifera* Dunel., not eaten.

I am indebted to Mr P. J. Greenway, Botanist to the Amani Research Station, for these identifications.

Height of flight.

In the majority of large flights that I saw the height of the swarm was from just above ground-level to about 100 ft.; occasionally the flight was concentrated in the lower 20 ft., or much thicker at this level than above. At other times the swarms were merely like brownish smoke high in the air, and one such swarm seen at Bura was estimated (by comparison of the apparent size of individual locusts seen through field glasses vertically and horizontally) to be well over 1000 ft. This particular swarm was in a state of violent turmoil, a spiral or circular motion being quite conspicuous even at that distance.

Speed of movement.

On various occasions the speed of flight of the adults, when flying without influence of the wind, was estimated at about 6–8 miles per hour, but no more exact observations were made.

At Naivasha in August, 1928, a number of observations on the speed of the hoppers was made with a stop-watch over measured distances. The hoppers were almost all in the last two stages of growth (IV and V).

There were two types of observations: (1) those on individual hoppers, and (2) those on the head of a column. All observations were made through field glasses from a distance.

A. Individual hoppers.

(1) Distance 6 yards along footpath, no obstructions, progress by walking only, no jumping. Times 55, 50, 45, 50, 50, 47 sec. Average time 49.5 sec. Average speed 438 yards per hour or slightly under $\frac{1}{4}$ mile per hour.

(2) Distance 5 yards by roadside, as above but different date. Times 50, 45, 45, 40, 45 sec. Average time 45 sec. Average speed 400 yards per hour.

(3) Distance 5 yards. Slightly cooler atmosphere. Times 60, 60, 80 sec. Average time 66.6 sec. Average speed 270 yards per hour.

B. Head of column.

(1) For 6 yards as A (1). Time 50 and 47 sec. Average time 48.5 sec. Average speed 445 yards per hour.

(2) Distance 5 yards, stopping continually to feed. Time $3\frac{1}{2}$ min. Speed 86 yards per hour.

(3) Between midday and 6 p.m. the head of one column had only advanced 60 yards.

It would appear from the above that about a quarter of a mile per hour is the maximum speed that is likely to be maintained by a column on the march and not feeding.

Natural enemies.

Birds. At Naivasha in August, 1928, large flocks of many thousands of birds flew into the hopper-infested areas each morning and flew back to their roosting grounds each evening. They appeared to follow definite routes in their daily movements, and flock after flock passed over Naivasha Hotel each morning as early as 6 a.m. returning about 5 p.m.

Two species were shot and were later identified at the Nairobi Museum as the Wattle Starling (*Perisornis carunculatus*) and the Superb Starling (*Spreo superbus*). In spite of their number they did not appear to affect materially the size of the hopper swarms.

Dipterous egg parasite (Stomatorrhyna lunata F.). On July 31st, 1928, I examined a small area near Naivasha, Kenya, where egg-laying was reported to have taken place. On digging in the soil the remains of old egg-masses were discovered and among them were two or three empty puparia of Diptera.

At Bura, Kenya, a large swarm of locusts commenced to lay about December 6th, 1928. I visited the locality on December 9th-10th when laying had almost finished. Small to large dipterous maggots were found in many of the egg-masses. In two cases there were over 30 maggots in one egg-mass and about 20 per cent. of all the egg-masses contained maggots. As the area covered by the laying extended over many miles the number of maggots must have been enormous, and one is tempted to speculate as to the origin of their parents.

No locusts had laid eggs in the district for at least 10 years and probably for 20 or 30 years, and yet within a few days of the arrival of a laying swarm of hundreds of millions of locusts, there was a supply of millions of parasitic larvae! The district round was most certainly typical grasshopper country, and perhaps the fly was normally a parasite of the resident grasshoppers. But even this would scarcely explain the way in which the supply was equal to the sudden demand, and the possibility of immigration has to be thought of, particularly in view of the observations given in the next section.

The parasitic dipteran were also seen in numbers in egg-masses at Moololo near Voi on December 12th. These eggs had been laid at an earlier date than those seen at Bura and many of the egg-masses had hatched, while the maggots had completed their development and

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pupated in the sandy soil between the egg-mass and the surface of the ground at a depth of about 1 in.

Flies bred from these larvae were identified by the Imperial Institute of Entomology as *Stomatorrhyna lunata* F.

In one case at Bura, Kenya, ants were seen carrying away maggots of these flies from the egg-laying areas.

Spheg aegyptiacus (Plate XXVIII, fig. 1). On January 29th the first swarm of locusts arrived at Amani, and three other swarms visited the Research Station between that date and February 7th, mostly coming from the north.

On February 18th a large swarm of locusts, still in the purple-brown stage, arrived more or less from the south and began to settle about 11 a.m. Within 15 min. I noticed numbers of a large black Sphegid wasp running about on the bare patches of the ground on roads, footpaths, tennis court, etc. These numbers rapidly increased and about midday my two laboratory assistants caught 168 specimens on an area of about 25 square yards in less than 1 hour, and there were as many about at the end as there had been at the beginning. This insect had never been seen by me during nearly two years' residence at Amani.

Almost immediately on arrival the Sphegids began to burrow in the ground, and a little later were seen to be dragging paralysed adult locusts along the ground and into their burrows. The *Spheg* stood over the head of the locust, seized the prey, usually by an antenna, near the base, and with rapid vibration of the wings ran across the ground to the burrow. This continued the whole of that day and even at 6.30 p.m. many wasps were still digging.

On the following morning (February 19th) the first *Spheg* was seen at 7.15 a.m. and they were active all morning, during which period there was a steady flight of locusts more or less to the north or north-east. Between 1 and 2 p.m. the number of locusts on the ground began to reduce rapidly and the general flight above began to rise higher up to 300 ft. At the same time the number of *Spheg* began to lessen rapidly and at 2.15 only four *Spheg* (one of which was dead) were seen on 100 yards of roadway, where two hours previously there had been thousands. The *Spheg* left in such a hurry that they left hundreds of open burrows, many half finished, and paralysed locusts were lying about in hundreds on the roadways, in some cases even just alongside the *Spheg* burrows. By 4 p.m. there was not a locust or a *Spheg* to be seen on the ground at Amani, although many thousands of the former were still passing overhead.

Evidence of the presence of the *Sphex* was found as far away as Sigi, about 2 miles east of Amani and 1500 ft. below; and at Kwamkoro about 4 miles south of Amani and at about the same level (3000 ft.).

A few days after this Mr Brown, Manager of Mavume Estate, about 6 miles to the north of Amani, came in to report that he had seen large numbers of "black bees" migrating in the swarms of locusts. He described them as being in large masses "about the size of a tree" which were passing low down with a loud buzzing sound, one lot passing so low and with such a noise over his head that he instinctively ducked to avoid them. There is little or no doubt that these were the *Sphex*.

Several other swarms of locusts settled at Amani in the following month, but in no case were they accompanied by *Sphex*. On the other hand on March 12th I visited Monga Estate, about 5 miles to the west of Amani, and found distinct evidence of the recent presence of locusts and *Sphex*, in the form of many abandoned burrows and paralysed locusts by the roadsides, and on the same day Capt. Nicholl, Manager of Kwamkoro Estate, 4 miles south of Amani, reported the arrival of swarms of locusts and *Sphex*, although 2 days previously there had been a large swarm of locusts without any *Sphex*.

The insect was identified by the British Museum of Natural History as *Sphex aegyptiacus*.

It would appear from the above observations that *Sphex aegyptiacus* has developed a migratory habit and accompanies the swarm of locusts on which it is preying. Nothing similar has been recorded so far as I am aware, but Mr G. Arnold informs me that he has noticed sudden increases in another species of *Sphex* in Rhodesia during locust infestations.

As long ago as 1857 Van Bemmelen (*Hand. Ned. Ent. Ver. (Leiden)*, iv, 81) records that a migratory flight of the butterfly *Pieris brassicae* in Holland on July 13th, 1855, was accompanied by a species of *Sphex* and *Musca vomitoria*, but I am not aware of any other record of migration in Sphegidae.

The observations given above have been briefly referred to by myself in (4) and (5).

Two or three other observations in connection with the *Sphex* appear worthy of record. In the first place all the specimens captured were females. No male was ever seen. Therefore either the species is parthenogenetic, or else pairing must take place before migration, and the latter instinct is not developed in the males.

In the second place although thousands of locusts were buried in the roads, footpaths and tennis courts on February 18th and 19th there was

no hatch out of *Spheg* in any of these situations up to the time of my departure on April 8th. After that time there was, unfortunately, no resident entomologist to take close observations, but other members of the scientific staff inform me that no hatch-out was ever observed. This might possibly be connected with the absence of males.

On February 18th I took away from the *Sphegids* six paralysed locusts and put them in a cage for observation. On the following day one was capable of jumping but fell on its side. On March 6th four were alive and two dead, on March 20 two were alive. On March 25th one was alive. On April 2nd all were dead. Thus two survived for over 4 weeks and one for over 5 weeks.

Minor enemies. At Naivasha, Kenya, on July 31st, 1928, the remains of an adult locust was found in the burrow of a larva of a tiger beetle (*Cicindelidae*). At Voi in December, 1928, a dead adult was brought to me which had been found with the abdomen pulled down into the ground by one of the ground-dwelling spiders. The discoverer had been under the impression that the insect was laying—a not unnatural mistake.

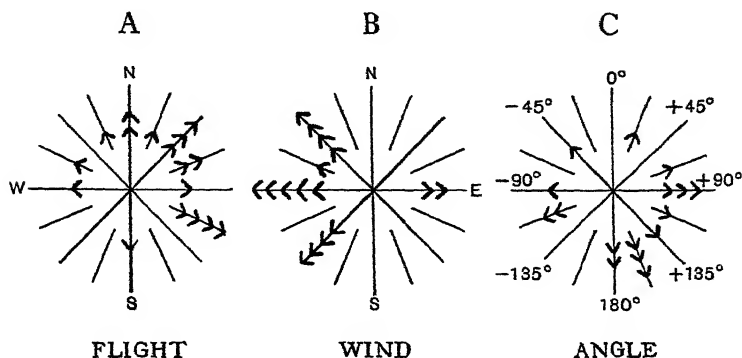
Orientation.

Definite orientation of locusts may be noted both when moving and when at rest.

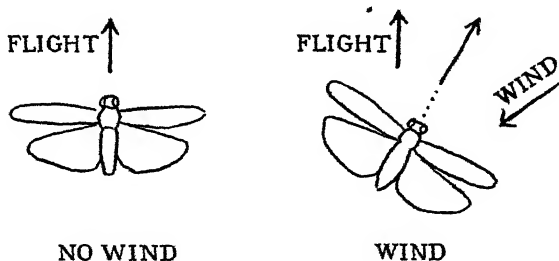
Orientation during movement is the determining factor in the direction of movement of swarms of hoppers, or flyers. Observations on locusts and on many other migrating insects have so far given no clue to the mechanism of the orientation. There appears to be no *regular* relation between flight and wind direction, although at times there appears to be a slight connection. When near the ground and particularly when alighting locusts tend to face the wind; this has the effect of reducing their speed relative to the ground. On one occasion I saw a small swarm of young flyers which were all flying with a fairly strong breeze at a level of 10–15 ft., but lower down at about 1–6 ft. all were flying in the opposite direction against the wind. All the time hundreds were rising from the lower to the upper layer or dropping, and all immediately changed direction as they changed their level. The locusts were probably near enough to the ground to be conscious by vision of the effect of the wind on their speed relative to the ground.

On seventeen occasions I recorded the direction of the flight and the direction of the wind. Text-fig. 1 A gives the flight direction, showing a distinct preponderance between north and east-south-east; Text-fig. 1 B

gives the wind directions (one calm), showing a distinct preponderance towards the south-west, west and north-west (*i.e.* north-east, east and south-east winds), while Text-fig. 1 C shows the angle between the flight and the wind in each observed flight. If the flight is directly with the wind the angle is 0° , if directly against the wind the angle is 100° , and if directly across the wind the angle is $\pm 90^\circ$ according to whether the



Text-fig. 1. Diagram showing observed directions of flight of desert locusts (A); of the wind at the time of these flights (B); and of the angle between flight and wind (C).



Text-fig. 2. Diagram showing relation between direction of wind, direction of flight and angle of body of locust.

flight is to the right or left of the wind. This latter figure shows a preponderance between $+90^\circ$ and 180° , *i.e.* across or against the wind, but with the small numbers of observations available it is impossible to say if this is significant or not.

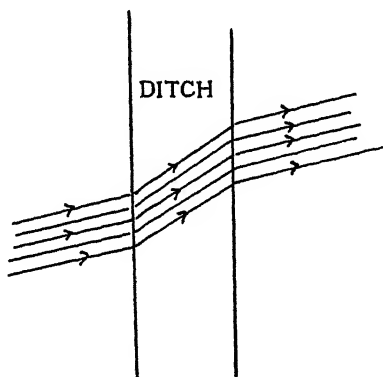
At times it was obvious on close observation that the locusts were turning their bodies at an angle to the line of flight when the wind blew strongly. Thus on November 29th at Voi, Kenya, I noted that when there was no wind the locusts were flying to the north-east with their heads

facing in this direction; when however the rather gusty wind blew from the south-east the locusts' heads turned approximately to the east although their motion relative to the ground remained to the north-east (see Text-fig. 2).

The orientation of moving swarms of hoppers on the ground according to my own personal observation seemed to be independent of external factors. Frequently it changed direction three or four times within an hour, and different parts of the same main swarm might be moving in quite different directions simultaneously. A sudden gust of wind (especially if dust laden) would cause a momentary change of direction, as also would a sudden fright.

I once watched a small swarm of hoppers attempting to cross a road; before they got well out on their journey they were turned back either by a sudden gust of wind or a passing motor car. After many attempts they gave it up and turned parallel with the road along its margin.

On another occasion I noticed that hoppers crossing a shallow dry ditch about 2 ft. wide changed direction slightly at the bottom of the ditch but resumed the normal direction when they got out on the other side (Text-fig. 3).



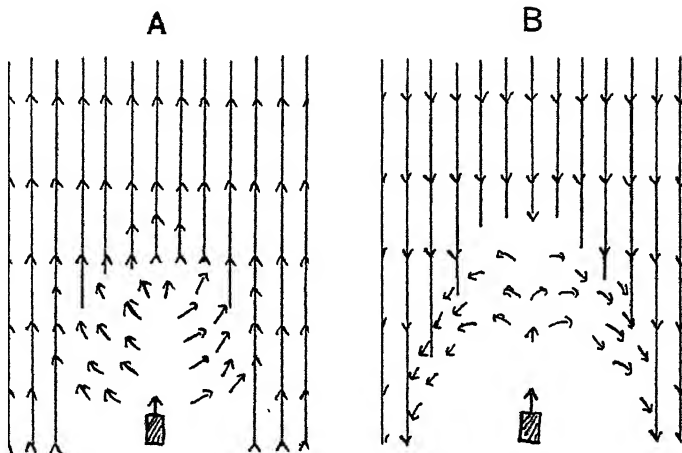
Text-fig. 3. Diagram showing slight change in direction of movement in a band of hoppers when crossing a shallow ditch.

Although hoppers were sometimes put off their direction by fright there were times when this was not so, and I noticed a distinct difference in their behaviour according to whether they were approached from the front or rear. If one walked through a trekking swarm of hoppers in the direction of movement they scattered to either side, but keeping more or less in the same direction (Text-fig. 4 A). If, however, one walked through against the direction of the trek, they spread in both directions at right angles and then resumed the original direction on either side (Text-fig. 4 B). A rapid approach created less permanent disturbance than a slow approach.

During sleep the locust makes every effort to be in a vertical position, head upwards—this usually results in them climbing up tree trunks, bushes or even grass stems, when dusk comes on. Moving swarms will turn towards any conspicuous object at this time, even to a standing

motor car or to a man if he keeps quite still. This ascent, however, is not essential as they will go downwards, as for example into the open mouth of an animal burrow, in order to get into a vertical position, head upward, on the sides of the opening.

During the day-time, when not on the move, orientation appears to be influenced by radiation. When the sun's rays are not too hot there is little definite orientation, but when the sun is shining strongly the adults place their body axis in the line of the sun's rays thereby reducing the amount of heat received (see p. 487). Under similar circumstances they will move into the shade of a house, but in this case I noticed on several



Text-fig. 4. Diagram showing direction of movement in a band of hoppers when disturbed by an observer going (A) in the same direction as the hoppers, and (B) in the opposite direction.

occasions that they go no farther into the shade than is absolutely necessary; thus there is a line of congregation just within the edge of a line of shade while there were few either in the sun, or further in the shade.

Relations of activity to climatic conditions.

The extent to which the sleeping, feeding and trekking activities of young and adult locusts are influenced by weather changes during the day is of considerable interest, and a fair amount of the available time was given to this study.

During the visit to the Rift Valley in August, 1928, three whole days (August 3rd, 7th and 11th) were followed through from daybreak

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to sunset and, in addition, a number of isolated observations were taken on other days.* The original intention had been to take dry- and wet-bulb temperature readings at short intervals during the day, but unfortunately the wet-bulb thermometer was broken during the first day's observations, so that only dry-bulb readings were available. It became more and more obvious that radiant heat played a distinct part in determining activity, but no black bulb in vacuum thermometer was available until the observations on swarms of adults at Amani in February, 1929.

A very brief summary is given below of the observations taken on two days on swarms of hoppers chiefly in the IVth and Vth stages at Naivasha (about 1° S. lat.), Rift Valley, Kenya. August 7th was a sunny day showing air temperatures up to 82° F. and great activity of the swarms; the 11th on the contrary was cloudy with the maximum temperature only 68° F. and the swarm very inactive.

Observations on swarm of hoppers, August 7th (sunny).

a.m.	Temp. ° F.	
6.5*	50	Swarm asleep; locust birds going out to feed.
6.37	53	Slight movement of legs of hoppers exposed to sun (thermometer in sun just above ground shows 57° F.).
6.47	54	Clear sky, no wind. Hoppers drop to ground if approached.
7.00	—	General wriggling but vast majority still on bushes.
7.20	58	Thermometer in sun 64° F., majority now on ground but many still up, especially in shade.
7.25	—	Fairly steady ground movement started to east, many feeding, still some not moved.
7.40	60	Slight trek to east, but soon stopped.
8.00	62	Most sitting about on grass, very few appear to be feeding.
8.25	64	Most sitting about, small trek started but possibly due to fright.
8.45	65	Small numbers beginning to cross road but vast majority sitting still, thermometer laid in dust in road reached 91° F.!
9.00	—	Bright sun, no clouds, very slight cool south wind; definite trek along and across road to north.
9.10	67	General activity under grass and along road.
9.15	—	General rustle of trek through grass, also along road; head of column frequently stopping, apparently to feed.
9.35	68	Dust in road 103° F.; trek along road ceased owing to fright of man passing; general movement in grass.
9.40	69	Trek in full swing all round; feeding on coarse grass.
10.20	70	Trekking and feeding; another attempt to cross road.
10.45	—	Temperature in dust on road 115° F.
11.00	72	Big trek down road to north-east; fair number feeding.
11.15	—	Numbers of adults flying north-east for a few minutes.
11.35	75	Trekking and feeding.
11.55	—	Clouds gathering; majority now feeding.
p.m.		
12.20	—	Trek along road has advanced 125 yards since daybreak; dust temperature 120° F.
1.5	77	Slightly cloudy; big trek down road; in grass most sitting up on stems but don't appear to be feeding.
1.40	75	Cloudy; trekking.

* All times are "sun time." The local "summer time" was 40 min. later.

p.m.	Temp. ° F.	
2.05	82	Bright sun; trekking.
2.25	77	Cloudy; wind south; main trek now to east; adults flying round.
2.40	73	Now about 175 yards from sleeping quarters.
3.10	67	Rain; little thunder; hoppers bunched into small bushes head up as if going to sleep; all trekking ceased; hoppers have even gone down into sides of holes as if for night; take practically no notice of my movements.
3.35	69	Rain over; hoppers crawling about and up a few stems of <i>Chenopodium</i> feeding; sun out and insects quite active and easily frightened; not trekking.
3.55	68.5	Cloudy; dust in road 71° F.; slight trekking.
4.35	69	Thin clouds; swarm to north of road have moved 30 yards since rain stopped and are now up feeding.
5.5	67	Most up, some feeding.
5.25	66	Dusky, cloudy; some still feeding, others settling for night.

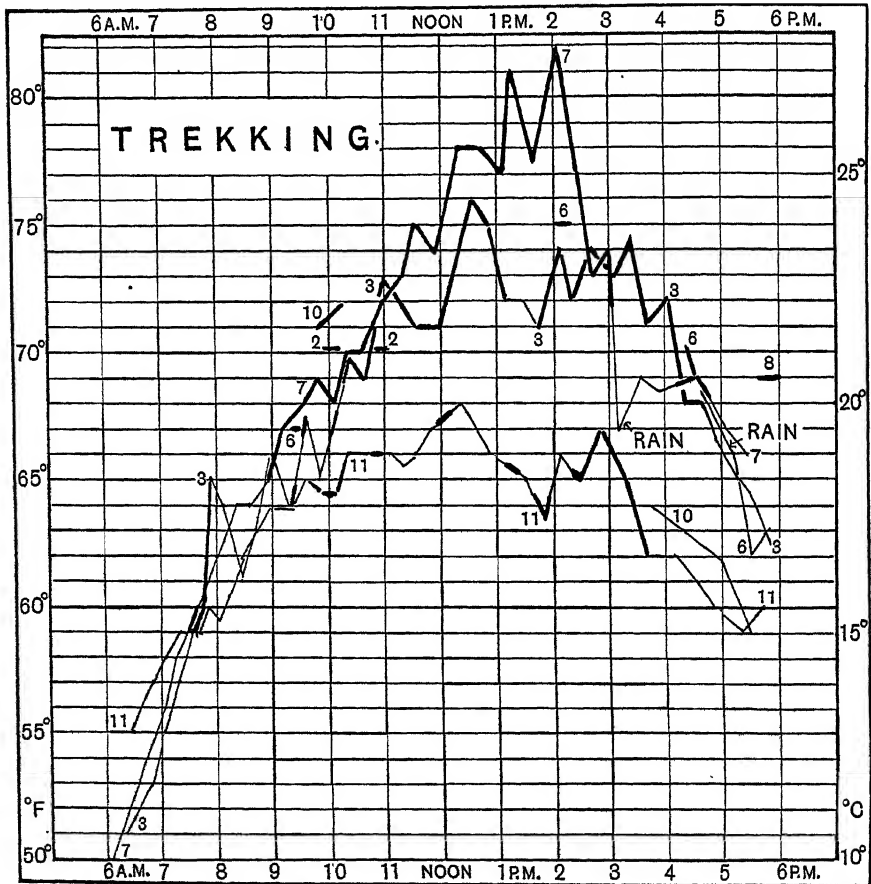
Observations on August 11th (cloudy).

a.m.		
6.05	55	
6.50	57	Cloudy; practically no movement.
7.20	59	No movement.
7.35	59	Thin clouds, slight rain, no movement.
8.05	59.5	Still cloudy; position of sun just visible; very slight movement.
8.25	—	Sun just visible but casts no shadow; slight activity, less than 10 per cent. down from sleeping quarters; slight leg movement is chief activity; occasionally one falls to ground.
9.00	64	Cloudy; no shadow; majority not moved.
9.35	65	Majority still in sleeping position; some excited over small heap of poison bran that I placed near them on ground; others quite near remained lethargic.
9.50	64.5	Still cloudy; slight trek through <i>Chenopodium</i> patch from which all cortex has been eaten.
10.20	66	Slight trek along road; many still in sleeping position.
10.40	66	Still a few up but most now on ground crawling round under <i>Chenopodium</i> ; slight trek to north, but most massed in heaps on ground and not sensitive to fear; have only advanced a few yards from sleeping place.
11.35	66	Still cloudy; many up resting or feeding; others massed on ground.
p.m.		
12.40	66	Bits of blue sky appearing but no direct sun.
1.30	65	All cloudy; slight trek on road; main swarm as before; sitting on stems or congregated on ground.
2.05	66	Sun beginning to shine through; in sunny moment adults seen flying north or north-east more or less with wind.
2.30	65	New trek along road to north-east; sun visible through cloud.
2.50	67	Sun shining; trek continuing.
3.05	—	Cloudy again; only slight trek.
3.40	62	Mostly cloudy; slight trek on road.
4.10	62	Getting up on bushes (? for night), some down holes; road trek ceased.
4.35	61	Majority up stems, settling for sleep; only a few on ground.
4.40	60	Practically all up; a very few feeding.
5.20	59	All up for night.

All the observations made at this period are summarised in the three diagrams (Text-figs. 5, 6 and 7). Text-fig. 5 deals with trekking activity from which it will be seen that trekking may start as early as 7.30 a.m. with an air temperature of about 59° F. On most of the days of observation it ceased before 5 p.m., but in the unusually warm night of

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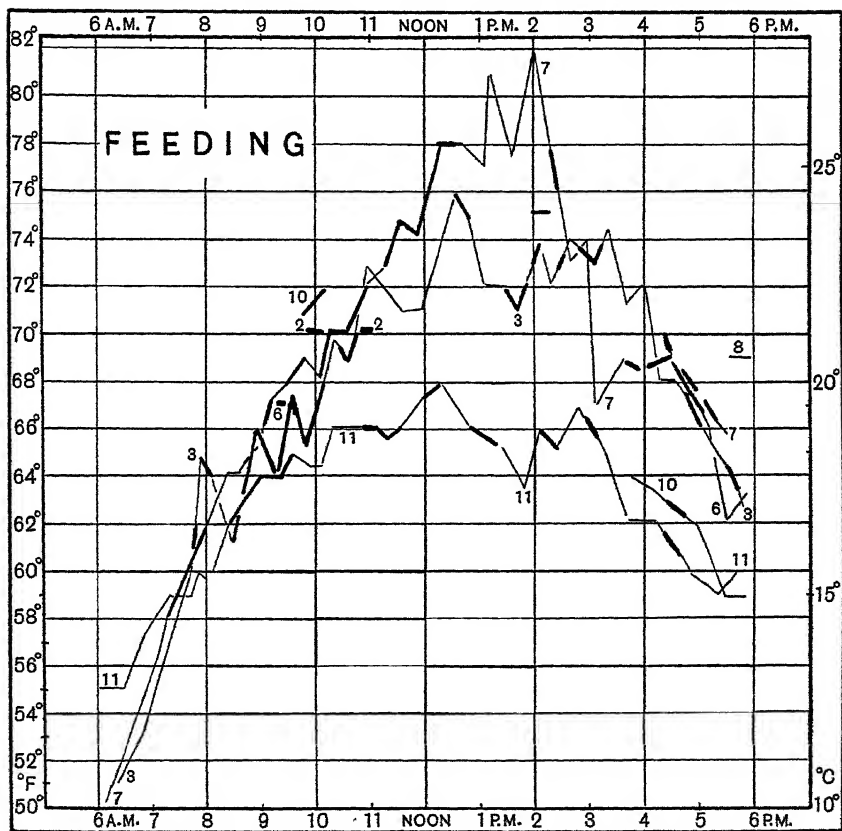
August 8th trekking was still taking place at 6 p.m. with an air temperature of 68° F. On the unusually cold August 11th trekking ceased before 4 p.m. at a temperature of about 62° F. With an air temperature above 68° F. (20° C.) trekking is practically always taking place except during rain or heavy cloud.



Text-fig. 5. Diagram showing the relation of trekking activity to temperature and hour of day on several days during August, 1928.

Text-fig. 6 deals with feeding and is much more indefinite than Text-fig. 5. Under the conditions of observation feeding appeared to take place at any time of the day when food was present and the air temperature above 60° F. It is, however, more general between the hours of 8-10 a.m. and 4-6 p.m. The locality under observation was dry rift valley pasture

with not much attractive food (judging from the way certain foods, such as the red cortex of *Chenopodium*, were eaten), and it is possible that the insects were always hungry. Most certainly bran was struggled for and eaten at any time of the day. If sufficient food had been present for the



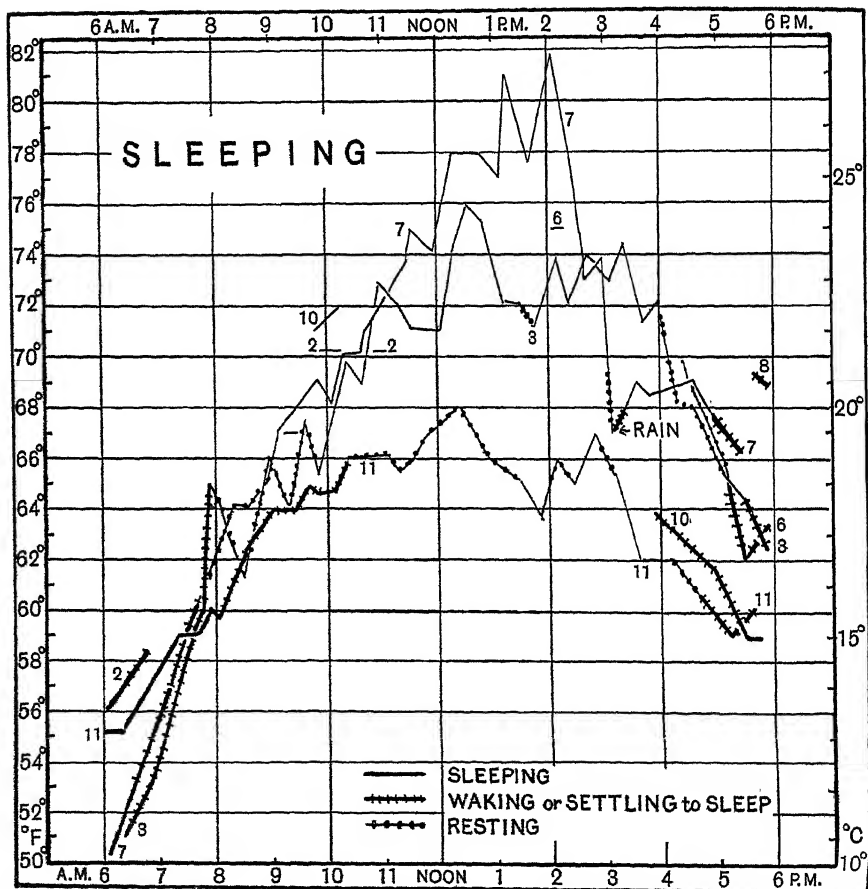
Text-fig. 6. Diagram showing the relation of feeding to temperature and hour of day on the same days as in Text-fig. 5.

hoppers to eat their fill in the first two hours of the morning it is possible that there would have been a more definite non-feeding trekking period in the middle of the day.

Text-fig. 7 deals with sleeping, waking or resting. The first signs of activity were usually seen about 6.30 a.m., but only in those subject to direct rays of the sun. By 8 p.m., unless very cloudy (e.g. August 11th),

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the majority were down and about, but some would be resting on the ground until 9-10 a.m. when the air temperature was above 66° F. Apart from cold cloudy days (11th) and cloudy or rainy periods on other days (3rd and 7th) there was very little cessation of activity till about



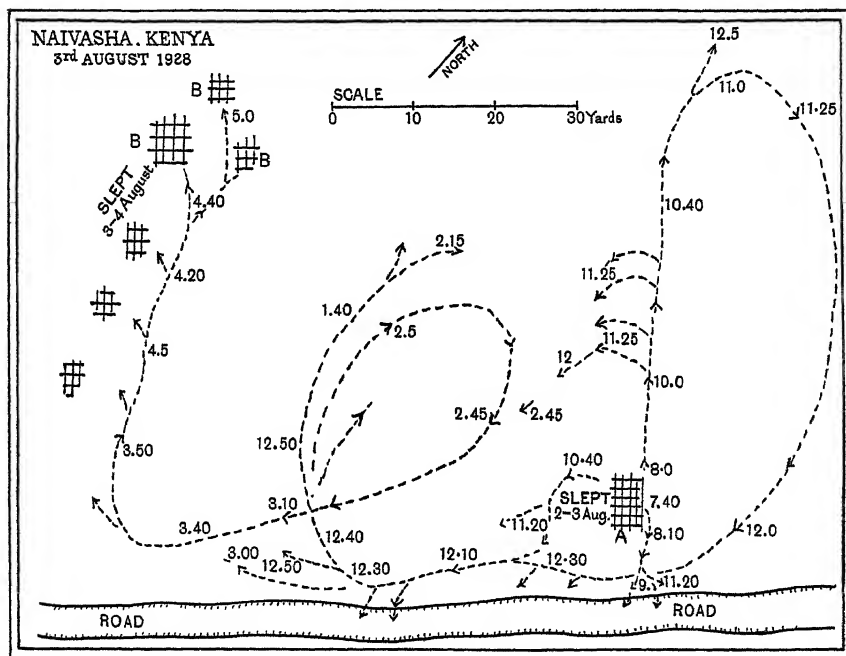
Text-fig. 7. Diagram showing the relation of sleeping and resting to temperature and hour of day on the same days as in Text-fig. 5.

4 p.m., when settling down appeared to take place independent of air temperature. Definite signs of going to sleep for the night were usually noted about 5 p.m. at temperatures varying from 59 to 68° F.

The results on the whole support the idea that there is a sequence during the day of sleeping—waking—feeding—trekking—feeding—

sleeping, but that it is liable to considerable modification due to weather changes and food supplies.

On August 3rd an attempt was made to follow out the movements of a small swarm of hoppers (chiefly stages IV and V) throughout the whole day. The results are shown in Text-fig. 8. The directional instinct was not anything like as definite as is generally recorded. It will be seen that, starting from sleeping quarters at A, an early trek started about 8 a.m. towards the road (south-east); nothing came of this but about 10 a.m.



Text-fig. 8. Diagram showing, approximately to scale, the actual movements of a swarm of hoppers during the course of one day at Naivasha, Kenya, on August 3rd, 1928.

the main body moved north-west and, after sending off a branch to the south-west, described a large circle to the right returning to near the sleeping quarters about midday; they then continued along the road to south-west and described another large circle to the right during the early afternoon. At about 4 p.m. they again veered to the right and took up sleeping quarters at B, about 75 yards in a direct line from the previous night's quarters. The actual distance travelled was, however, about 350 yards. Various small side off-shoots which apparently led to nothing or joined up with the main body are shown in the diagram.

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Every effort was made not to disturb in any way the direction of the main swarm by frightening them. Most observations were made by means of field glasses, often from the roof of a closed motor-car. On several occasions, however, the hoppers made attempts to cross the road and were frightened back either by passing cars or pedestrians or by sudden gusts of wind.

Observations on flying locusts were only made on one occasion when a large swarm of adult purple-brown locusts arrived at Amani on February 18th, 1929, and settled for the night. The following is a summary of observations taken together with the temperature (both air and black bulb) and humidity records.

a.m.	Air temp. ° F.	Re- lative humi- dity %	Black bulb	
6.45	67	95	—	Sun weak through clouds on horizon, locusts not yet moving.
7.00	—	—	91	Frightened off one or two locusts with saw and file (see p. 488); locust moving in cage in sun.
7.15	68	91	99	First <i>Sphex</i> seen at work.
7.35	69	87	107	Small numbers (hundreds) in the air flying chiefly south-west to north-east; those on ground nearly all orientated same way; numbers in air increasing every minute.
7.44	—	—	—	Already thousands on the wing but not 1 per cent. of those present.
8.05	71	83	118	Stream of tens of thousands in air, chiefly to north-east.
8.20	72	79	115	Hundreds of thousands going mostly to north-east, all below 100 ft.; but still vast majority on trees.
8.45	73	76	118.5	Passing in millions like snow; view indistinct, ground orientation distinct in some places, not in others; wasps busy.
9.00	—	—	128	Steady flight to west-north-west; wind north-north-east, sun east-north-east; still sufficient numbers resting to make trees purple.
10.15	77	65	132	Still passing in millions, settling also on bare ground; also many on trees but fewer than an hour ago.
11.45	80	59	137	As thick as ever; air, ground and trees all full.
p.m.				
12.50	82	52	139	Thicker and faster; those on ground show tendency to get into shade, especially just inside shadow line of house; those in sun tend to face direct into sun by elevating front end, or by resting on upright grass or twig.
1.20	80	59	115	Cloudy; thicker than snow, those on ground flat.
1.30	—	—	—	Sun out again; black bulb 129° F. and rising; those on ground with head, or more rarely tail, pointing to sun.
1.35	—	—	140	Orientation of locusts on grass plot very definite.
1.45	—	—	—	Flight much higher, up to 200 or 300 ft.; all wasps gone; on 100 yards of road only saw three alive and one dead.
2.15	81	60	120	Cloudy; as thick as ever up to 200 ft. at least; flight more northerly.
2.45	80	69	128	Millions mostly about 40 ft. up; view obscured; those on ground mostly flat.

p.m.	Air temp. ° F.	Re- lative humi- dity %	Black bulb	
3.00	—	—	—	Nearly all gone off ground, still thick in air and a few on trees.
3.10	78	72	120	Settling in enormous numbers on opposite side of valley about a mile or two away.
3.30	77	71	109	All locusts left ground and trees at Amani and great reduction in those passing in air.
3.50	—	—	—	Numbers in air rapidly diminishing, only a thousand or two in sight.
4.10	75	76	104	Only about 100 locusts visible in any one direction; going north.
5.45	—	—	—	Occasional locust seen flying.
6.15	72	84	70	Just getting dark; locusts all gone.

It is impossible to draw conclusions from a single day's regular observations, but it appears that locusts began to be active about 7.30 a.m. with an air temperature about 70° F. and black bulb just over 100° F. They were in full activity during the morning with black bulb between 110 and 140° F., and air temperature between 70 and 80° F.

When the black bulb reached 130° F. or over the locusts on the ground showed an increasing desire to reduce the radiant heat absorbed by orientating to face the sun. At black-bulb temperature below 120° F. this was not seen.

Amani is a damp hill station and the relative humidity did not fall below 50 per cent.

FIELD EXPERIMENTS.

Perception of sound and movement.

It was generally believed by planters that swarms of flying locusts could be frightened away, or at least prevented from landing, by getting men and boys to run up and down beating tin cans. It was obvious that two factors might be concerned in the "frightening," namely movement and sound.

Previous experience in watching locusts had made it clear that the insects were very sensitive to sudden movements even without sound, but it remained to be shown to what extent sound without obvious movement could disturb them.

The arrival of a swarm of purple-brown adults at Amani on February 18th, 1929, gave the opportunity for some experiments along these lines.

(1) A powerful intermittent alarm clock was placed on the ground in the midst of a swarm of resting adults. The alarm continued to go off

for periods of 15 sec. at 30 sec. intervals for about 5 min. Flyers within a few inches of the clock and some actually resting on the clock itself made no visible movement (as seen by powerful field glasses from a distance of about 10 yards) when the noise stopped or started.

(2) An electric buzzer, similar to an electric bell, but only producing a buzzing noise, had also no visible effect.

(3) A small tent was built of an umbrella and several blankets. Within this hiding place tin cans were beaten and guns fired when the tent itself was thickly covered with flyers. An observer at a short distance outside the tent reported not the slightest visible effect on the locusts.

(4) Standing as still as possible in the midst of a thick swarm, until the locusts were used to my presence (they were actually crawling over my boots), I fired a 12-bore shot gun down into the ground. Except for those disturbed by flying particles of dust kicked up by the shot the locusts, even within a foot of the muzzle of the gun, made no apparent movement. This was repeated several times with the same results.

(5) An electric motor horn (New Ford) was blown at irregular intervals. A few locusts leapt into the air at the first sound, quite obviously disturbed by it, but after a few repetitions no further effect could be obtained. The locusts appeared conscious of the sound, but rapidly became used to it.

(6) An ear-splitting noise was produced by a hidden assistant drawing a heavy triangular file along the edge of a large rip saw. Hundreds of locusts in the vicinity immediately leapt into flight, disturbing others, and in a few seconds perhaps 50 per cent. of the locusts within a radius of 10 yards were in the air. A repetition of the noise brought a smaller proportion of the remaining locusts into the air, and further repetition produced still slighter disturbances. Finally there were left a quite considerable number of insects which appeared to be quite unaffected. An examination was made of these to find if both sexes were still present (i.e. if one sex had been more sensitive to the sound than the other). Twenty-two males and twenty-eight females were found in the residue, a proportion similar to that in the original swarm (see p. 465).

This last experiment was repeated a number of times, always with the same result.

One would infer from these preliminary experiments that better results could be obtained in "scaring" locusts by a large-toothed metal "policeman's rattle" than by producing sounds, such as the beating of tin cans, which they apparently were unable to hear.

It was intended to extend these experiments with electrical apparatus

to find the range in pitch and intensity of their hearing powers. As I was unable to do this perhaps some other investigators will think it of sufficient interest.

Railway clearance.

At Naivasha in the Rift Valley in August, 1928, on a few occasions the railway trains were stopped when ascending inclines by the presence of hoppers on the lines. The hoppers were crushed and formed a slippery mess on the lines on which the driving wheels could get no grip.

In the Bura-Voi district in December, 1928, the yellow adults were settled in millions on some parts of the railway track for pairing and egg-laying. Time and again the trains were stopped when ascending a slight incline by these being crushed by hundreds under the driving wheels, the wheels slipping round and producing a most unpleasant smell of singed locust fat.

I spent some time studying this question and travelled many miles lying on the front buffers of trains, experimenting with brushes and watching the behaviour of the locusts. It was found that the oncoming trains frightened the locusts on the ground between the lines and that they leapt outwards in thousands. The majority were stirred into activity by the immediate approach of the front of the engine, and in their leaps they frequently reached the line between the front bogie wheels and the driving wheels. For this reason brushes attached to the front of the engine failed to prevent the slipping of the wheels. In fact it was surprising how few locusts would cause slipping on an incline.

As it seemed impossible to prevent the locusts being frightened, experiments were tried to frighten them sooner so that they had time to escape. The following method was found to work (for winged locusts). When the driver saw the track ahead covered with locusts on an up-hill he slowed off and three boys with branches or flags in their hands ran ahead, one about 30 yards, one about 20 yards and one about 10 yards in front of the engine, and boys and train advanced at a smart run. The first boy startled the locusts, the second boy caused them to hop and jump violently sideways; by the arrival of the third boy they were pouring outwards and the track was clear for the train. On reaching the top of the incline or the end of the swarm the boys jumped on board and the train gathered speed.

It may be thought somewhat undignified for a train to advance at the speed of a man's run, but it was better than not advancing at all, which was often the alternative.

No opportunity occurred to try any special mechanical fitments to the engine, but it seems possible that brushes in front of the wheels would work if they were combined with some downwardly projecting plate on the *inside* of the wheels to prevent locusts leaping outwards between the driving wheels and the front bogies.

Direct control.

Although my instructions were to concentrate on biological work and leave direct control measures to the local Department of Agriculture, it so happened that we had available at Amani one or two poisons that the local Department had not tried, and with their co-operation a few experiments were carried out.

Calcium cyanide. A swarm of hoppers, chiefly in the IVth and Vth stages), sleeping in fairly long grass, were dusted with "Cyanogas" calcium cyanide dust in the early morning (6.40 a.m.) of August 2nd, 1928, at Naivasha, Kenya.

All the locusts fell to the ground at once, and it appeared at first as if they had been killed. There was, however, no way of keeping up the concentration of the gas, and within 2 hours the locusts had revived and were on the move, and not a single dead locust could be found.

Poison bran experiment. In the same locality some experiments were carried out with poison bran spread before the advancing swarms of hoppers.

(1) As there was some idea that the locusts only fed in the morning and evening (but see p. 483), bran was spread about midday in a narrow line across a road along which the hoppers were advancing. They crowded on to the bran struggling to reach it, and fed readily. It is perhaps necessary to repeat that the country was dried up and suitable food not too plentiful, so that it is possible that in the regular early morning feeding time they had been unable to satisfy their hunger.

(2) A bait was made up containing 1 lb. arsenic to 5 lb. bran, to see if the arsenic had any deterrent effect. It was eaten quite readily.

(3) Small heaps of poison bran were laid in front of an advancing swarm. The heaps were of the following composition in addition to the bran:

- (a) Sodium arsenate.
- (b) Sodium arsenate + orange juice.
- (c) Sodium arsenate + almond essence.
- (d) Sodium fluosilicate.
- (e) Sodium fluosilicate + orange juice.
- (f) Salt.

After a number of observations it was impossible to say that any one heap was more or less attractive than the others.

(4) On August 8th, at 2 p.m. a small handful of poison bait made up of bran and sodium fluosilicate, was put into two cages, the first containing 47 adults and the second containing 30 stage V and 45 stage IV hoppers.

The death rate was as follows:

Started August 8th, 2 p.m.	Adults	Stage V	Stage IV
August 9th, 7.30 p.m.	16	10	15
August 10th, 1.0 p.m.	7	6	8
August 11th, 9 a.m.	18	9	12
Alive at end of 3 days	6	5	10

Percentage death-rate in 3 days: Adults 83, Stage V 83, Stage IV 77.

(5) On August 9th, at 10.30 a.m. hoppers were collected in the field where they were gathered in crowds round two heaps of bait, one poisoned with sodium fluosilicate and the other with sodium arsenate. They were confined in cages with the following results:

	Arsenate	Fluosilicate
August 9th, 10.30 a.m. number captured	48	76
August 10th, 9 a.m. dead	9	3
August 11th, 9 a.m. dead	19	32
8 p.m. dead	10	20
Alive after 2½ days	10	21

Percentage death-rate after 2½ days: sodium arsenate 79, sodium fluosilicate 71.

From the above experiments and a number of general observations I came to the conclusion that in the semi-arid type of country which was then infested in Kenya, the poison bran method gave one of the best promises of control for the larger hopper stages, and that sodium fluosilicate was practically as valuable as arsenic as a poison.

A few remarks might possibly be made on the general question of poison bran.

For use against a swarm of hoppers which is passing across an area more or less in one direction it is necessary to have a thicker spread than for non-migratory grasshopper control, as the latter wander backwards and forwards over any area and have more opportunities of finding the bait.

Gibson (*Agric. Gazette, Canada*, II, 10) records that by using 20 lb. bran and 1 lb. of arsenic spread over 5 acres of land he obtained an average kill of 406 grasshoppers per square yard. This means then an average of 22 grasshoppers were killed for each milligramme of arsenic

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in the bait, or roughly one grasshopper for each milligramme of bait. I examined 100 mg. of bran and found it to consist of approximately 300 pieces of material (most very small), so that in the above case only three particles of bran were responsible for the death of one grasshopper. It would be interesting to compare the weight of different locusts and grasshoppers and the minimum fatal dose of poison.

LABORATORY OBSERVATIONS.

Effect of isolation on phase coloration.

On December 11th, 1928, just hatched black gregarious hoppers were collected at Mbololo near Voi, Kenya, and were brought back to Amani. Then they were put into breeding cages $26 \times 22 \times 42$ cm. or approximately 24,000 c.c. capacity, two sides glass, three sides wire and one side wood (see p. 495). In some cages 50 larvae were put and in others only two hoppers. It was noticeable that there was very much greater activity among the crowded locusts than among the isolated ones.

Conditions at Amani did not seem to be favourable for breeding purposes, and a very heavy mortality followed. All hoppers, whether in crowded or isolated conditions, remained in the swarm coloration of black, dark green and yellow, and at the end of March the only two survivors, both in the last nymph stage, had each been in complete isolation in separate cages for over three months and yet they showed not the slightest trace of transformation to the paler green solitary phase.

These preliminary experiments are by no means conclusive or on a large enough scale, but they most certainly show that isolation does not invariably lead to the production of the solitary type of coloration.

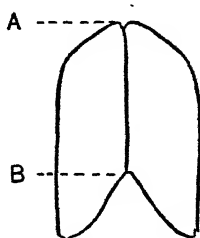
Development of sexual maturity.

An important problem in the biology of desert locusts is the change that takes place in the adult insect at the time of sexual maturity. This change is shown externally by the altering of the colour from a purplish brown to bright yellow, and internally by the rapid development of the ovaries and testes at the expense of the fat body which is correspondingly reduced.

A number of dissections were made which are given below. Unfortunately most of them are for immature locusts and a few for those just showing the beginning of the change from brown to yellow, and only one in the fully developed yellow stage. At the only opportunity that I had for studying the mature stage in November and December,

1928, I was so busy with other problems that the dissections had to be postponed.

The ovary of the female locust is a conspicuous feature of the anatomy and is easily seen as soon as the dorsal wall of the abdomen is removed. When small or partly developed it is of the shape shown diagrammatically in Text-fig. 9. The measurements given below are from *A* to *B*. The ovary is already well developed in stage IV female larvae, as dissections 8 and 9 will show.



Text-fig. 9. Diagram showing system of measurement of size of ovaries.

It is necessary to emphasise once more that the dissections were part of a preliminary investigation and were never intended to be a complete account.

Dissections made at Naivasha, Kenya, in August, 1928.

August 2nd	(1)	Female adult, age unknown but quite young; purple-brown; ovaries 18 mm. long.
	(2)	Female emerged to adult previous day; ovaries about 9 mm. long.
August 4th	(3-5)	Females 1 day old; each ovary about 11 mm. long.
August 5th	(6)	Female dissected after boiling in water and keeping in alcohol; not so good as first but ovaries quite visible, 14 mm. long.
	(7)	Stage V larva; ovary quite distinct, 4.5 mm. long.
	(8)	Stage IV larva (young); ovaries not distinct.
	(9)	Stage IV larva (older); ovaries about 3.3 mm. long.
August 6th	(10)	Female, age not known; ovaries 9.5 mm.
	(11)	Female, age not known; ovaries 10.5 mm.
	(12-14)	Females 1 day old; ovaries each 11 mm. long.

Locusts received at Amani, September 27th, from Kenya.

- (15) Female, purple, age unknown; 11 mm.
- (16) Female, purple, age unknown; 13 mm.

Dissections at Voi, November 1928.

November 29th	(17)	Female, yellow; large ovary with fully developed eggs nearly filling whole body.
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Dissections made at Amani, Tanganyika, in January and February, 1929.

January 31st	(18)	Female, purple-brown; ovaries 13 mm.; very little fat.
	(19)	Female, purple-brown; ovaries 14 mm.; very little fat.
February 20th	(20)	Female, purple-brown; ovaries 11 mm.
	(21)	Female, purple-brown; ovaries 10 mm.
March 5th	(22-23)	Female, purple-brown; ovaries 10 mm.
	(24)	Female, purple-brown; ovaries 10.5 mm.
	(25)	Female, purple-brown; ovaries 9 mm.
	(26)	Female, purple-brown; ovaries 9.5 mm.

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- March 8th (27) Female, legs still pink, base of wings and abdomen just beginning to turn yellow; ovaries large, 14 mm. long and deep orange in colour; very little fat.
- (28) Female, base of wings slightly paler, possibly also abdomen; general colour not so vivid, but earlier stage than (21); ovaries still cream coloured, 9.5 mm. long; more fat than (21).
- (29) Female, much as (22), but base of wings more distinctly yellowish; ovaries orange, 15 mm. long; little fat.
- (30) Male, wing slightly yellow at base, general colour paler; abdomen distinctly yellow except at base; hind-legs distinctly yellowish; little or no fat body; testes yellowish, about 7 mm. long.
- (31) Male as above but abdomen only yellowish in outer half and beneath, reddish above; hind legs missing; little or no fat body; testes as (24), but damaged in dissection.

It would appear from the above that the ovaries are about 9–13 mm. long when the female becomes adult and that they remain in about this condition until the change from purple to yellow takes place. After this they must enlarge rapidly, but unfortunately I was not able to get sufficient material of this stage.

Weight of adults.

On February 19th, 1929, weighings were made from a swarm of purple-brown immature adults that arrived at Amani.

	g.
26 females weighed	59.8
36 males weighed	67.7
Average: female	2.30
male	1.88

On August 10th, 1928, at Naivasha, Kenya, it was found that 17 adults (not sexed) weighed 1 oz. This gives just over 600,000 to the ton, or to compare with the above an average weight of 1.68 g. These adults had only recently changed from the last nymph stage.

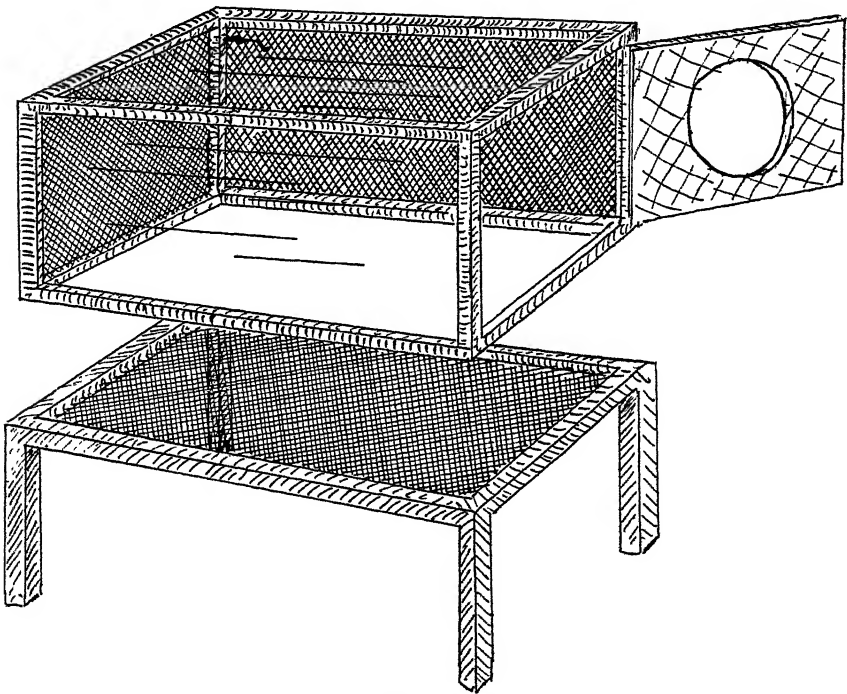
Unfortunately no opportunity occurred for weighing yellow mature adults.

TECHNIQUE.

Observation of swarms in the field.

The hopper stage of the desert locust, as has been shown above, is extremely sensitive to the movement of objects in its vicinity except at low temperatures or during rain or heavy cloud. This makes it a difficult insect to observe closely. Whereas a caterpillar or even such an active insect as a wasp may often be slowly approached and observed from a distance of a few inches, locust hoppers on the contrary cease their normal activities or alter their direction of movement at the slightest movement or approach of an observer. Their sense of sight must be unusually well developed.

At first I found it very difficult indeed to find whether or not feeding was going on in some of the swarms. In many cases, however, I was able to settle such questions by observing the swarm from a distance of about 10 yards with a pair of high-power prism field glasses. These were invaluable. Had I returned to East Africa I intended to take with me one of the Davon microtelescopes to see if these would be capable of greater use owing to their much higher magnification.



Text-fig. 10. Breeding cage used for isolation experiments.

Later, however, I found that there was another way of observing the habit of the hoppers, and that was to approach *quickly* instead of slowly. By running into the middle of a swarm, then lying down at full length as rapidly as possible and then keeping perfectly still, I found that the initial momentary fright of the hoppers appeared to pass off when no further movement occurred. They would then resume what they had been doing without delay. By this means I have watched the hoppers feeding within a few inches of my eyes and had them "trekking" over my legs. So long as I kept quite still I was merely part of the landscape.

Breeding cages.

In view of the fact that I had a very high mortality in my experimental cages, it seems scarcely policy to recommend to others the cages I used. However, as I now believe that my losses were chiefly due to keeping the cages underneath the shade of a verandah, in a district which was already too damp and sunless for the locusts to breed well, I will describe the cages briefly for the help of other workers.

The cages were made 42 cm. long, 22 cm. high and 26 cm. wide, the floor, one side and one end was made of copper wire net, the top and one side of glass and the remaining end was a wooden door in which was fitted a round hole and a sleeve (Text-fig. 10).

The floor was raised up on short legs about 10 cm. high, and the legs and floor were separate from the rest of the cage which could be lifted off for cleaning purposes. This was also done so that other types of bases (e.g. deep sand for egg-laying) could be placed under the same cages.

SUMMARY.

The paper deals with observations made on the desert locust in East Africa in 1928-9. Two broods were observed to develop between May, 1928 and April, 1929. Females slightly outnumbered the males in a number of random collections of adults. A list of plants eaten or avoided by the adults is given including species from 62 natural orders, of which 18 are represented by both edible and inedible species. Notes on the food plants of the hoppers are also included. Hoppers of the IVth and Vth stages were observed to have a maximum speed of about $\frac{1}{4}$ mile per hour.

The chief natural enemies seen were *Stomatorrhyna lunata*, a Dipterous parasite of the egg, and *Sphex aegyptiacum*, a predator on the adult locusts. The latter had developed a migratory habit and followed its host.

No regular relation between direction of flight and direction of wind was observed.

Trekking activity in swarms of hoppers of the IVth and Vth stages was most noticeable when the air temperature was above 15° C.

Feeding was most general when the air temperature was above 10° C., and in the swarms observed, which were probably short of food, took place at almost any hour of the day.

Continual observation of one band of hoppers throughout a whole day showed that the direction of movement was by no means constant. In fact two complete circles were made in the course of the day.



Fig. 1.

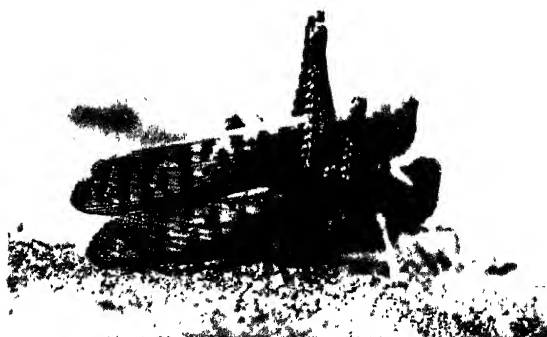


Fig. 2.

WILLIAMS.—OBSERVATIONS ON THE DESERT LOCUST IN EAST AFRICA FROM
JULY, 1928 TO APRIL, 1929 (pp. 463-497).

Adult locusts were found to orientate head towards the sun when the black-bulb temperature was 130° F. or above.

Adult locusts were found to be insensitive to sounds such as the banging of tin cans (often used for scaring purposes) or the firing of a gun, but were much frightened by the noise of a file drawn down the edge of a saw.

Railway trains were got through swarms of egg-laying adults on the ground by having several boys running in front of the train at short intervals. This procedure frightened the locusts off the line and was much more successful than any system of brushes in front of the wheels.

A few small-scale experiments with poisoned bran showed no difference in effectiveness between sodium arsenate and sodium fluosilicate.

A few individuals from a swarm of just hatched hoppers were kept in isolation until the last larval stage, but did not assume the pale colouring of the solitary phase.

Dissections of adult females showed that the ovaries remained small (about 11 mm. long) as long as the locusts remained purple-brown in colour.

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EXPLANATION OF PLATE XXVIII.

Fig. 1. *Spheg aegyptiacus*, predacious on desert locusts in East Africa.

Fig. 2. Male of desert locust on the back of a female while the latter is in the act of egg-laying. Notice the abdomen of the female buried in the ground almost to the base.

(Received November 16th, 1932.)

A CAMBIUM MINER OF BASKET WILLOWS (AGROMYZIDAE) AND ITS INQUILINE GALL MIDGE (CECIDOMYIDAE)

BY H. F. BARNES, M.A., PH.D.

(*Entomology Department, Rothamsted Experimental Station.*)

(With Plates XXIX and XXX and 12 Text-figures.)

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I. INTRODUCTION AND METHODS.

THE following paper deals with the morphology and bionomics of an interesting Agromyzid fly which mines or tunnels in the cambium of basket willows. In addition, an inquiline gall midge is described for the first time and compared with another gall midge also new. The bionomics of this inquiline are given.

I am indebted to Mr T. Justin Cowley, who first showed me the larvae of this fly, for much assistance in this work. He has supplied me

both with material and information regarding the habits of the larvae. Mr H. P. Hutchinson, willow officer at the Long Ashton Research Station, has aided me considerably by discussing the problem and also supplying me with sets of the different willows used in the experiments. I am also grateful to Dr A. D. Imms, Dr C. B. Williams and Mr H. C. F. Newton for their help in diverse ways. Prof. F. Hendel, Mr J. E. Collin and Dr Ferrière have very kindly helped me with the identification of the fly and the parasites. In addition I should like to place on record my thanks to many correspondents, especially Mr H. McClelland of the Seale-Hayne Agricultural College. Mr V. Stansfield is responsible for the photographs.

Pupae were found in the first instance by sifting three sacks of soil taken from a space of 2 ft. square by 11 in. deep around an infested willow stub. From this amount of soil seven pupae were obtained. Larvae were collected by cutting rods in an infested area in July, August and September and then peeling the rods in the laboratory. Adult flies were caught in willow beds at Batford at the end of May and in early June.

For mating and oviposition, the flies were placed in glass muslin-covered cylinders which were stood over tubes containing willow shoots in water. When it was desired to breed from the flies they were put into muslin cages which had been placed over growing willow plants in 10-12 in. pots in an unheated greenhouse. In this way the complete life cycle was followed.

Some larvae were mounted directly in De Faure's fluid, others after being killed in boiling water and cleared with potash were stained with Ziehl's carbol-fuchsin and mounted in balsam.

II. IDENTIFICATION AND HISTORY.

The Agromyzid in question belongs to the genus *Dizygomyza* Hendel (subgen. *Dendromyza* Hendel) and is very closely allied to the two species *carbonaria* Zett. and *cambii* Hendel. The following notes are taken from a letter written by Dr Hendel:

"The four specimens (♂ ♀) kindly sent to me by Dr Barnes belong to the genus *Dizygomyza* Hendel (subgen. *Dendromyza* Hendel). The synopsis of the species in Hendel's Agromyzidae, p. 18 (in Lindner's *Die Fliegen der palaearktischen Region*, No. 59, 1931) runs to number 9 a. Neither *Dizygomyza cambii* Hend., nor *carbonaria* Zetterstedt *sensu* Hendel can be the same species as the fly of Dr Barnes, for both lack the postero-dorsal bristles on mid-tibiae.

"The single specimen of *Dizygom. cambii*, bred from *Salix* twigs by Prof. de Meijere in Holland has not haired eyes; but it has 6-7 orbital-

bristles on the front and 10-11 irregular rows of acrostichal hairs between the dorso-central bristles on the mesonotum.

"*Dizygom. carbonaria* (Zett.) Hend. has the third *dc* bristle in the middle between the second *dc* and the suture of the mesonotum, while the third dorsocentral bristle of *cambii* and of the fly of Dr Barnes is distinctly approached to the mesonotal suture.

"Barnes' fly has the haired eye of *carbonaria*, but the position of the *dc* bristles as in *cambii*.

"Only an examination of Zetterstedt's types of *Agromyza carbonaria* in Lund may make a correct determination of these species possible."

Dr Hendel has very kindly written a description of this fly¹ for me and this is included in section III(d) of this paper.

The history of cambium miners is limited and dates back to 1868 when Ratzeburg described some larvae found in the wood of birch trees in 1853 as *Tipula suspecta*. Previously Hartig in 1851 had described the damage². For some time, however, writers considered the "fleck marks" or "medullary spots" as one of the specific characters of forest trees. However, Kienitz (1883) made an important addition to the literature and came to the conclusion that the medullary spots of various deciduous trees were occluded channels which had been mined in the cambial cylinder by insects. One of his plates is reproduced as fig. 3 on Plate XXX. Grossenbacher (1910) gave a useful summary of the literature on the subject, as did also Brown (1913) and Record (1911). Nielsen (1906 *a* and *b*) was the first to rear the adults of these mining larvae and discovered them to be *Agromyza carbonaria* Zett. Actually he obtained the adults by rearing out pupae which he had found at the base of some alder trees. Then as *A. carbonaria* considerably exceeded in size all the other species of *Agromyza*, except *A. lappa* whose larval habits were already known, Nielsen came to the conclusion that *A. carbonaria* was the adult of the larvae to be found in alder, hazel, birch, willow, mountain ash, *Pyrus* and *Prunus*. It is exceedingly doubtful if it is the same species whose larvae attack such a wide range of trees. Nielsen described its life history and also summarised the literature on the subject. The next species to be reared and so definitely associated with fleck marks was *Agromyza pruinosa* Coq. which Greene (1914) ascertained was the cambium miner of river birch in America. Another species was reared from *Prunus avium* and *P. domestica* by Grossenbacher (1915) who

¹ Dr Hendel has proposed the name *Dizygomyza barnesi* sp.n. for this fly.

² The damage noted were discoloured spots in the wood and are variously described as "fleck marks," "Markflecke," "pith-ray flecks," "pith flecks" and "medullary spots."

described it as *Agromyza pruni* Gross. This was redescribed by Malloch (1915). Two further species were discovered in America and described by Greene (1917) as *Agromyza aceris* Greene and *Agromyza amelanchieris* Greene. In Europe a further species was reared by de Meijere (1925) from *Salix* and described by Hendel (1931) as *Dizygomyza cambii* Hendel.

The species discussed in this paper may therefore be considered as the third European species to be investigated, while three other species have received attention in America.

Mr Collin has suggested to me that the cambium miners to be found in other trees (besides willow) such as birch belong to different species.

III. MORPHOLOGY.

(a) *The egg.*

The egg (Text-fig. 1) is cylindrical with the posterior extremity broadly rounded whilst the anterior end is more pointed. It is opaque white and measures about 0.85 mm. in length and just over 0.2 mm. at its widest point. The micropyle at the cephalic pole is very obvious and is enshrouded by a supposedly gelatinous covering which protrudes beyond the anterior end of the egg in a comb-like structure. The chorion has a reticulated surface as indicated in the figure.

(b) *The larva.*

General and cuticular processes. The general character of the larva is shown in Text-fig. 2. It is opaque white, long and narrow, reaching a length, when full grown, of 18 mm. with a width of 1 mm. When first exposed in its burrow by peeling off the skin of the willow rod, the larva appears flattened, dorso-ventrally compressed, and greatly elongated, but it immediately assumes its correct tubular shape and contracts in length.

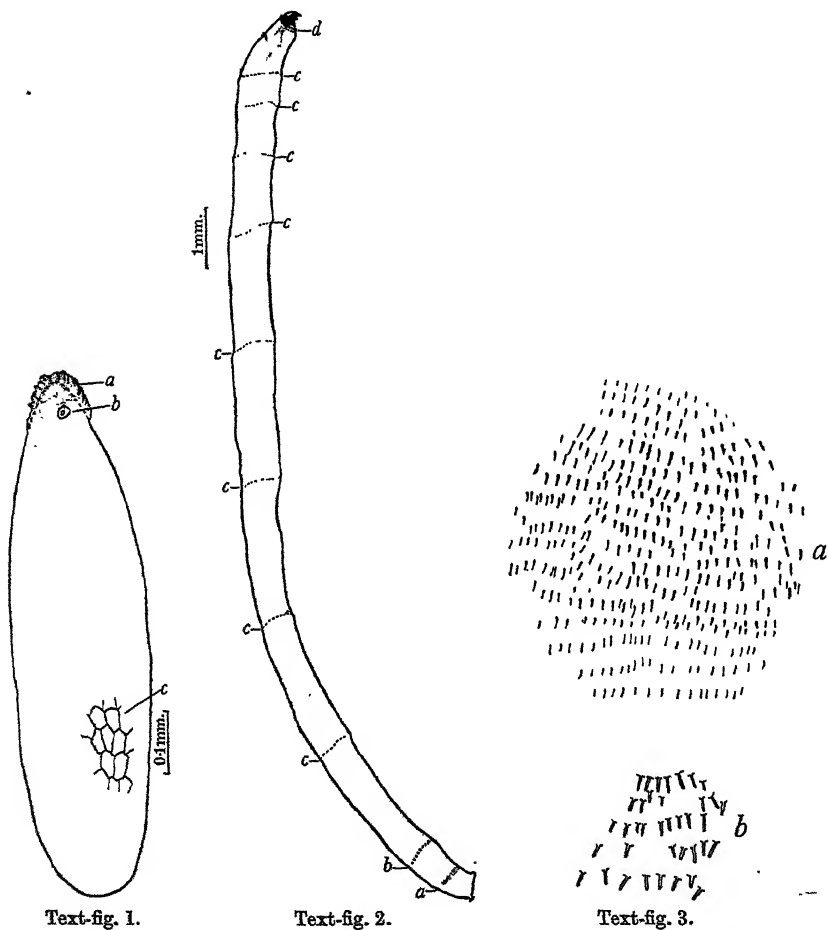
There are two types of cuticular processes, (a) those on the prothoracic segment, and (b) those on the segmental lines. Both sets of processes aid the forward movement of the larva in its burrow or mine and retards its movement backwards.

(a) On the prothoracic segment there are numerous microscopic chitinous finger-shaped processes (Text-fig. 3), which are directed backwards. They are arranged in about thirteen roughly parallel rows which form a collar at the anterior end of the segment.

(b) The second type is illustrated in Text-fig. 4. They are brown, chitinous and scale-like in appearance, directed posteriorly and arranged in rows. Starting with the anal segment, on each side at about one-eighth

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of the segment anterior to the posterior spiracles are 4-5 rows of such structures (*a*), then at about three-eighths of the segment are two such



Text-fig. 1. The egg. *a*, gelatinous covering; *b*, micropyle; *c*, indication of surface recticulation. $\times 83$.

Text-fig. 2. The larva. *a*, *b*, *c*, rows of scale-like cuticular processes; *d*, collar of finger-shaped cuticular processes. $\times 8$.

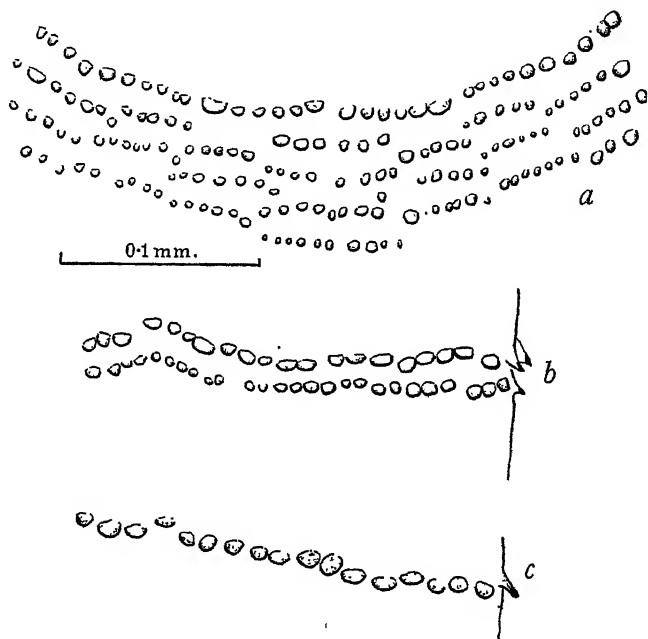
Text-fig. 3. Portions of the collar of finger-shaped cuticular processes. *a* $\times 385$, *b* $\times 600$.

rows (*b*) which extend completely round the body except for a small broken space ventrally. Anterior to these, corresponding roughly to the intersegmental spaces, are eight single rows (*c*) of similar processes.

These single rows appear to encircle the body. For the position of the rows see Text-fig. 2 (*a*, *b* and *c* correspondingly).

Greene uses the most posterior rows of these cuticular processes to separate the larvae of *Agromyza aceris* Greene from those of *A. amelan-chieris* Greene.

The cephalo-pharyngeal skeleton (Text-fig. 5). The mouth-hook (*m.h.*) is single and has a large median apical tooth (*a*). On each side there are

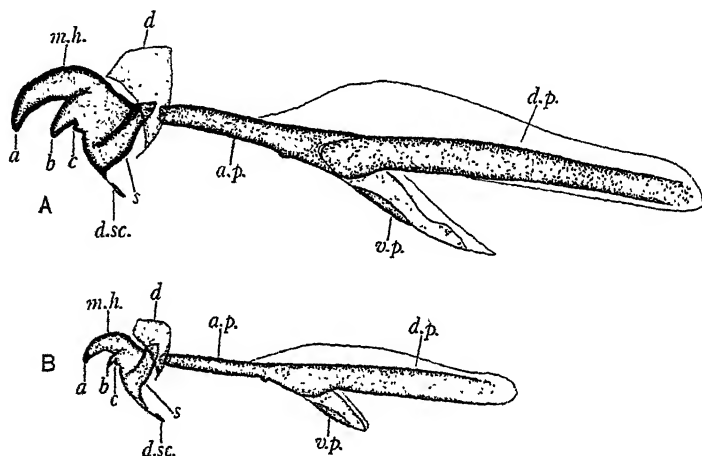


Text-fig. 4. Rows of scale-like cuticular processes. *a*, posterior set of rows; *b*, double row on the anal segment; *c*, single row on anterior segments of larva. $\times 275$.

two unequal smaller teeth (*b*, *c*). This single mouth-hook and large median apical tooth must be the result of a fusion of the two usual mouth-hooks. Hendel (1931, p. 6) states that two mouth-hooks are always present and figures those of *Dizygomyza postica* Meig. in his Text-fig. 14. The posterior portion (*s*) of the mouth-hook is separated from the anterior part, which bears the teeth, and forms a shield on either side of it, being joined to it at the base only. A slightly chitinated piece (*d*) is situated by the side of the mouth-hook and the anterior process of the pharyngeal sclerite. Below the ventral surface of the mouth-hook there

is a small elongate piece which is probably the dentate sclerite (*d.sc.*). The antennal organ is situated immediately above the median apical tooth of the mouth-hook. In the last instar the anterior process of the pharyngeal sclerite (*a.p.*) appears to be separate from the dorsal process (*d.p.*) and the ventral process (*v.p.*), whereas in the penultimate instar there is complete fusion.

The spiracles. In the penultimate and ultimate instar the larva is amphipneustic. The anterior spiracles are situated on the dorsum of the prothoracic segment just posterior to the collar of cuticular processes.

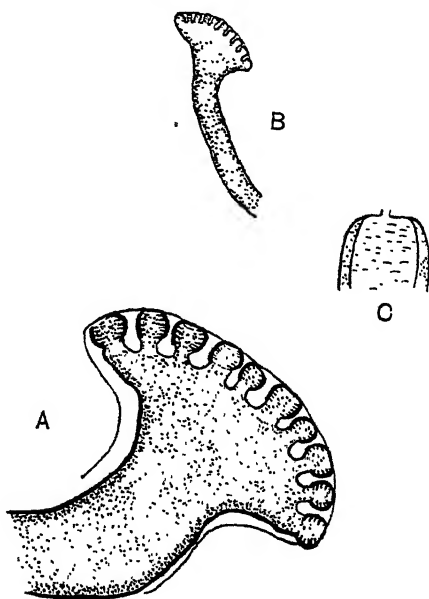


Text-fig. 5. Cephalo-pharyngeal skeleton. A, last instar larva; B, penultimate instar. $\times 275$.
a, apical tooth; b, c, lateral teeth of mouth-hook; a.p. anterior process of pharyngeal sclerite; d, chitinised piece; d.sc. dentate sclerite; d.p. dorsal process of pharyngeal sclerite; m.h. mouth-hook; v.p. ventral process of pharyngeal sclerite; s, posterior portion of mouth-hook.

In lateral view they are rosette-shaped with a varying number (8–12) of cup-shaped bulbs. The openings are minute pores situated on a slightly raised projection (Text-fig. 6). Viewed from above, the spiracles are long and narrow showing one opening on each bulb. In colour they are pale yellow and are slightly raised above the surface.

The question of the exact number of cups or bulbs is interesting. In the penultimate instar (of which unfortunately only one specimen was obtained by actually seeing the moult), there are 10 such cups on one side while there appear to be 11 on the other. In the last instar of which many specimens have been examined the number varies most definitely. In 10 specimens the variation is as follows: 9 and 10 (the spiracle which

has 9 cups has the 5th and 6th cups fused), 8 and 11, 10 and 11, 10 and 10 (one spiracle has the 8th and 9th cups fused), 9 and 10, 9 and 10, 10 and 11, 10 and 12, 10 and 11, and 9 and 11. It has not been possible to see whether the lower number of cups is constantly situated on the spiracle of one side or the other. This variability in number throws doubt upon their use as diagnostic characters of the different instars, especially as in the one specimen of the penultimate instar the number appears to be the same as in the last instar. Steel (1931), working on the frit-fly,



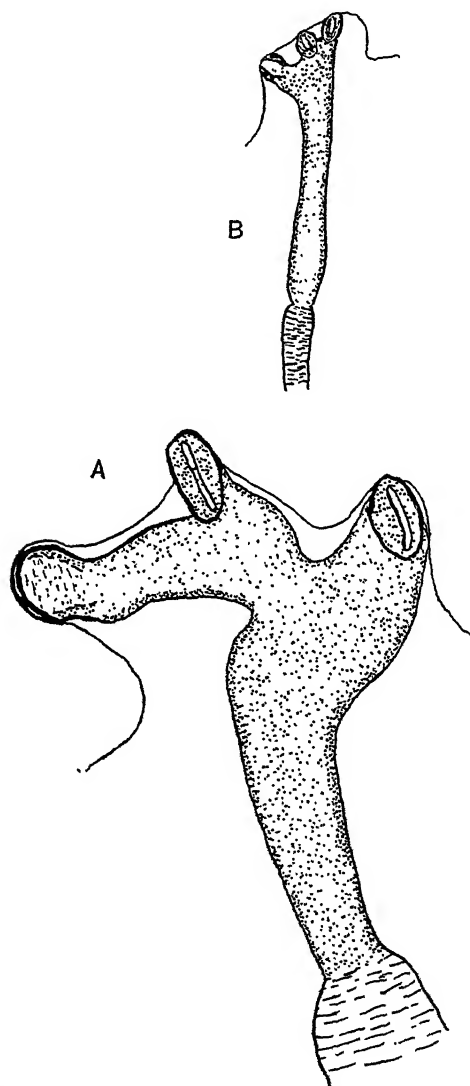
Text-fig. 6. Anterior spiracle of: A, last instar larva; B, penultimate instar larva; $\times 578$.
C, diagram of single bulb of spiracle showing aperture.

found that the normal number of digitate processes of the third instar larva was 6, although in some cases there were only 5 as in the second instar larva.

The posterior spiracles (Text-fig. 7) are pale yellow and have three chitinised plates each with a long narrow slit. The three plates are asymmetrical, one being borne on a longer arm than the other two, while the stigmatic chamber leads off from these latter two plates. In both the penultimate and last instar the number of the plates is constant and is three. This is in direct contrast to the observations of Nielsen (1906 *a*),

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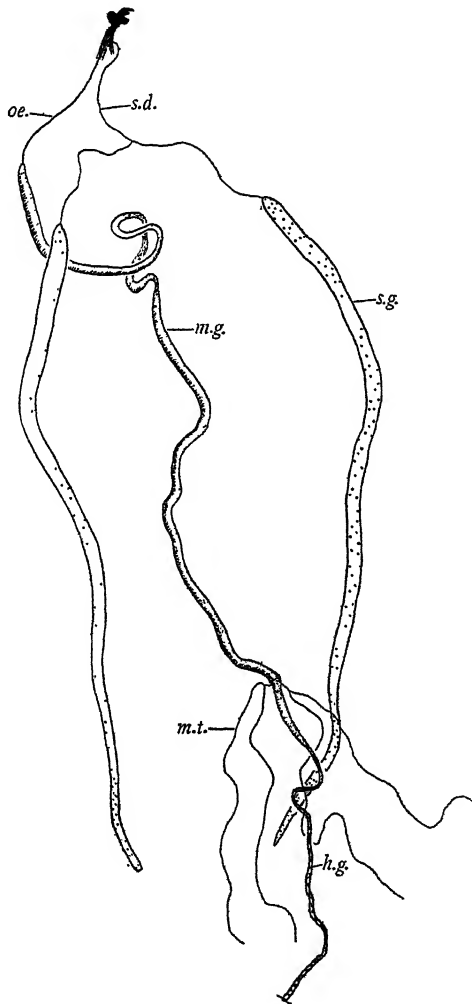
who figures the posterior spiracle of the first instar as having one plate, the second instar two and the third instar three plates.



Text-fig. 7. Posterior spiracle of: A, last instar larva; B, penultimate instar larva. $\times 578$.

The digestive system (Text-fig. 8). The alimentary canal is divided into three easily recognised regions: (a) the fore-intestine, (b) the mid-intestine and (c) the hind-intestine.

The oesophagus is a single narrow tube being only about twice as large in diameter as the salivary duct. The stomach or mid-gut is a



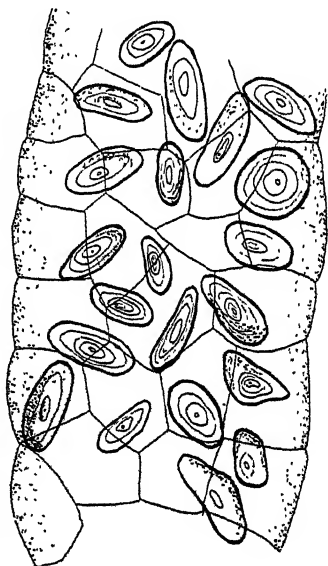
Text-fig. 8. Digestive system of larva. *h.g.* hindgut; *m.g.* midgut; *m.t.* Malpighian tubules; *oe.* oesophagus; *s.d.* salivary duct; *s.g.* salivary gland. $\times 8$.

long wide tube which is slightly coiled. The hind-intestine starts at the insertion of the Malpighian tubes, but at this point and for some little distance it is of the same size as the mid-gut. Hence the small intestine is not very well differentiated from the colon. More posterior lies the

colon which is of smaller diameter than the small intestine. The rectum is poorly defined.

The excretory organs and fat-body. I. The Malpighian tubes (Text-fig. 8). There are two pairs of Malpighian tubes, each pair becoming fused just before entering the alimentary canal.

II. The fat-body (Text-fig. 9). The fat-body is very conspicuous on account of the calcospherites which it contains. The latter are exceedingly numerous and must number several hundreds at least. Keilin (1921) figures the larva of an *Agromyza* species containing 120 such bodies and the writer has a specimen from lettuce leaves containing about 60-70. The calcospherites consist of calcium carbonate, and this storage of a waste product is typical of a large number of Diptera which are either parasitic, phytophagous or living in putrefying substances. A good account of this phenomenon is given in the above-mentioned paper by Keilin. One of his conclusions may be quoted with advantage. "During the first days of metamorphosis the calcium carbonate dissolves in the perivisceral fluid (haemolymph or blood of insects) and then passes through the newly formed pupal cuticle into the ecdysial fluid. When the latter is absorbed, the calcium carbonate remains as a deposit upon the internal surface of the puparium."



Text-fig. 9. Portion of fat-body, showing calcospherites. $\times 115$.

It has been observed in the species under consideration that the newly formed pupa contains the calcospherites but that older pupae do not. Also that the empty puparia are lined with a whitish powdery substance which effervesces on coming in contact with dilute acid. These observations endorse Keilin's conclusion of ecdysial elimination as quoted above.

The glands or organs of secretion. Labial glands (Text-fig. 8). The labial glands or salivary glands are very prominent and large. They are of normal structure, being paired and their ducts uniting to form a common duct. The cells of the glands are large and would be ideal for histological study.

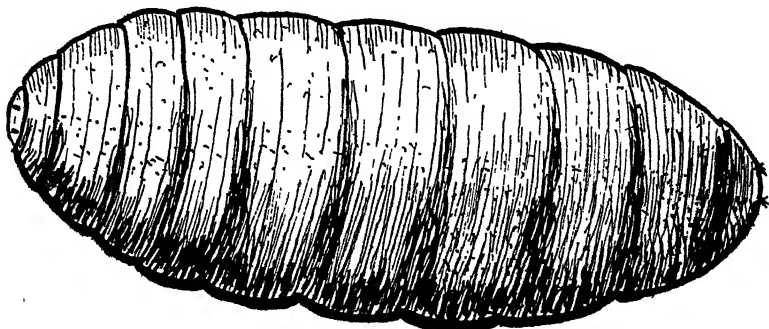
(c) *The pupa.*

The pupa (Text-fig. 10) is of normal coarctate shape, the puparium being cylindrical, dark reddish brown and about 4–5 mm. in length. Both the anterior and posterior spiracles are inconspicuous.

(d) *The adult, Dizygomyza barnesi sp.n. Hendel.*

The adult (Plate XXIX, figs. 1, 2 and 3) has been described for me by Dr Hendel as follows:

“Dr Barnes’ fly (*Dizygomyza barnesi* sp.n.) may be distinguished in the following manner. Width of front almost one and half that of eye, as long as broad; width of each orbit (behind) about one-sixth that of frontal stripe; 4–5 orbital bristles, the hindmost bend backwards; parafacials broad, *not smaller below than above*; height of cheeks equal to about one-fourth the eye height; eyes scantily haired. Mesonotum with



Text-fig. 10. Pupa. $\times 25$.

3 + 1 dorsocentral bristles; the third appreciably nearer to the suture than the second *dc*; the praescutellar pair between the first pair of *dc*, not before it as in *carbonaria*; 8–9 irregular rows of acrostichal hairs. No praesutural intra-alar bristle as in *carbonaria*. One or two postdorsal bristles on mid tibiae, while *carbonaria* and *cambii* have no distinguishable bristles there. The colour of the whole fly is black; frontal stripe opaque, orbits and ocellar triangle slightly glossy; mesonotum, scutellum and abdomen with greyish pollinosity, only slightly shining. Sixth abdominal segment elongated; base of ovipositor shorter than the 6th segment, almost cylindrical, transversely wrinkled. In all other respects the fly resembles *Dizygom. carbonaria* (Zett.) Hend. Length $3\frac{1}{2}$ –4 mm.”

Dr Hendel possesses a male and a female which I sent him, while I am in possession of another male and female which were also examined by him. These specimens were obtained from material collected in Hertfordshire in 1931.

IV. DISTRIBUTION.

This insect is common in basket willow beds at Mawdesley near Ormskirk, Lancashire, and it occurs in willow beds at Batford near Harpenden, Hertfordshire. Specimens have also been received from Beccles, Suffolk (through Ministry of Agriculture) and from near Selby, Yorkshire.

Search has been made for its occurrence by willow growers in Devon, Essex, Leicestershire, Lincolnshire and Somerset, but without success.

Inquiries have been sent to growers in other districts of England, Ireland, Scotland and the Isle of Man, but without result.

It seems probable, however, owing to its wide distribution, that it will be reported from other districts when it becomes better known.

V. BIONOMICS.

(a) Emergence, mating and oviposition.

Emergence of the adults takes place at Harpenden from about May 21st until the end of June. The flies remain alive for about a week in captivity.

Mating has only been observed once. No signs were seen of any courtship and actual coition lasted from 8.30 a.m. until 1.45 p.m. (standard time). Egg-laying started in this case at 2.30 p.m. the same day.

When about to oviposit the female walks up and down a willow stem and usually chooses a point about 10 in. to a foot from the ground on a young shoot in its first year of growth. Having selected the spot, the fly bunches up its abdomen and puts its ovipositor at right angles to the plant epidermis; then it starts boring into the stem. This proceeds with a steady pushing motion and the fly moves backwards slightly as the ovipositor sinks into the tissue, until the abdomen is again horizontal to the surface of the stem. Then it remains perfectly still for an instant while the egg passes down the ovipositor into the stem. The ovipositor is withdrawn and the insect generally flies away to another shoot before repeating the process. A single egg is laid in each puncture. A small hole in the epidermis is all that is visible, the egg being placed right in the tissue with only the anterior end penetrating into the inner layers of the epidermis.

(b) Damage.

The damage done is caused by the larvae tunnelling up and down the stem in the subcortical layer or cambium. The larvae confine their activities to the basal portion of the rods, the stub and the roots. Thus

when the rods are grown for sets the most valuable portion is useless. Similarly for basket work the thick end of the rods has to be thrown away. As plant growth continues these tunnels become covered by new tissue. The result is that the rod is weakened throughout. This becomes of greater importance when the rods are grown for stake rods, *i.e.* for 2 years or more. Finally, if the willow were to be grown as a tree for wood it would be useless owing to the so-called fleck marks which are scattered throughout any cross-section. In Plate XXX these various types of damage are illustrated. Fig. 1 is a slightly diagonal section of a 4- or 5-year-old set of *Salix viminalis* and shows the fleck marks. Fig. 2 is the cross-section of a 2-year-old rod of Long Skin variety of *S. viminalis*. The rod was tunnelled slightly in the late summer or autumn growth of the first year, very badly in the early growth of the second year and slightly again in the second autumn growth. Fig. 4 is the same rod in longitudinal view, when peeled, and shows a tunnel exposed. The two smaller white objects are the cocoons of the gall midge inquiline which is to be described later in this paper. The larger white object in the burrow is the larva of the fly.

Besides the damage done by the actual tunnelling, the tunnels themselves are points exposed to bacterial and insect attack. This frequently takes place. In such cases rot quickly sets in and the stems soon have an appearance very similar to normal attack by black canker. Plate XXX, fig. 5, shows the external appearance of a rod primarily attacked by the fly and secondarily infected with rot. Fig. 6 on the same plate shows the appearance of the rod when partially peeled. In both these figures as well as in figs. 2 and 4 (Plate XXX) cavities can be seen caused by the secondary infection. It is probable that black canker itself may follow the primary fly damage.

The easiest way of detecting the presence of this cambium miner is to look for cankerous rods and then cut the rod in order to see whether the typical fleck marks as seen in Plate XXX, figs. 1, 2 and 3(1) are present or not. Alternatively one can peel the rods at their bases in order to expose the tunnels, but this is only possible at those seasons of the year when the tunnels are near the surface. Except in cases of secondary canker and very severe attack, when there may be several larvae burrowing in a single rod, there is very little change in the external appearance of the growing rods. Sometimes, however, there is a slight discoloration and sinking inwards of the skin immediately over the burrow. The damage is most noticeable in August and September and when the rods are peeled.

(c) *Host plants.*

The most usual species of basket willow to suffer attack is *Salix viminalis*, of which the varieties Dark Long Skin, Greenskin and, to a less extent, Continental are prone to damage. In addition, larvae and tunnels have been found in Lancashire in commercial beds on the following varieties and species: Pomeranian (*S. triandra*), Dicky Meadow (*S. purpurea*), Harrison (*S. viminalis* × *purpurea*) and Black Top (*S. triandra* × *viminalis*). In Yorkshire it has been found on *S. viminalis* chiefly and also on *S. purpurea*.

In greenhouse experiments at Rothamsted it has been successfully reared on Long Skin (*S. viminalis*). Attempts have also been made to rear it on Dicky Meadow, Black Maul (*S. triandra*) and Cricket Bat Willow (*S. caerulea*). In the case of the last-named species, the adults were observed laying their eggs very readily, but the larvae died before reaching maturity. This was almost certainly due to failure on the part of the writer to keep the plants in good condition during the hot weather of July, 1932. The females used in the experiments on Dicky Meadow and Black Maul failed to lay eggs; this, however, was probably due to the fact that only old females were available. Field records however have shown that Dicky Meadow (*S. purpurea*) and at least one variety of *S. triandra*, viz. Pomeranian, are liable to attack.

In view of the peculiar interest of its being able to oviposit and live on Cricket Bat Willow, search has been made where possible for its occurrence on this species in the field. But so far it has not been found for certain although the writer has been told of one case where fleck marks, which might have been due to this insect, were supposed to have been seen.

Apparently this fly prefers soft wooded varieties to hard ones.

(d) *Life cycle.*

The egg stage lasts from about a week to a fortnight. As soon as the larva hatches, it starts mining downwards towards the stub, remaining throughout its life in the cambium tissue. Its passage can be traced by the fine tunnel it leaves immediately below the skin. Its progress is irregular but there is a downward tendency for the greater part of June and July. Cast skins can be found in the tunnels which appear to sink inwards as fresh plant growth takes place and covers the old passage. The tunnelling has been found right down to the tips of the smaller roots but never more than about 3½ ft. up the rods. Towards the end of July the larvae change their direction and start working upwards. When ready to pupate the larva makes a slit in the outer skin of the rod (see

Plate XXX, fig. 3) and, coming out, gets to the soil. It has not been observed exactly how this is accomplished. It appears probable that the larvae either crawl down the outside of the rods in damp weather or, if it is too dry for this, remain on the rod near the exit holes until they have become much shorter in length preparatory to pupation and then drop to the ground. The exit holes may be found up to about 3 ft. up the rods but frequently occur in the bottom foot. They are quite characteristic. It is possible that some of the larvae make exit holes on the stubs but none have been found in this position or on the roots. These slits or exit holes have been found as early as July 21st, while larvae have been found as late as September 16th. Usually, however, most of the larvae come out of the rods during August.

Pupation takes place in the top few inches of soil around the stubs and the puparia remain here throughout the winter and spring. They have been found as early as July 28th on occasion.

Emergence of the adults occurs the following May and June. There is only one brood a year.

VI. PARASITES.

The following Braconid parasites have been reared: *Symphia ringens* Halid. and *Symphia hians* Nees (Lancashire and Hertfordshire). Both these parasites are illustrated in Plate XXIX, figs. 4 and 5. A further Braconid, *Apanteles fulvipes* Halid., is provisionally associated as a possible parasite of the *Dizygomyza*.

It is interesting to note that Greene (1914) records *Symphia agromyzae* Rohwer as a parasite of *Agromyza pruinosa* Coq.

Parasitism by the *Symphia* species appears to be quite high. Adequate numbers have not been reared to be certain of this, but from Lancashire material in 1931 three flies and one *Symphia* were reared; in 1932 from material collected in the same locality six *Symphia* only were bred. From Hertfordshire material, sample A gave seven flies and four parasites and sample B five flies and three parasites, both in 1932.

However, the parasitism is not sufficient to prevent the flies being numerous enough to cause very prevalent damage in these two localities. Sometimes hardly a rod of Dark Longskin is free from attack, in other years 50 per cent. of the rods are tunnelled.

VII. INQUILINE GALL MIDGE, *PROFELTIELLA DIZYGOMYZAE* SP.N.

The larvae of this gall midge live as inquilines in the burrows of the cambium miner. The adult midge is very distinctive in coloration, the wings being bright yellow mottled with black, the legs banded with yellow and black and, in the case of the female, the abdomen bright red. It is also of moderate size.

Two species in this genus have previously been described, firstly *P. ranunculi* (Kieffer) which was originally placed in the genus *Lestodiplosis* (Kieffer, 1909). Later Kieffer (1912) raised the genus *Profeltiella* for this species. The larvae of *P. ranunculi* were stated to feed on the larvae of another gall midge, *Geodiplosis ranunculi* Kieffer, which live on the roots of *Ranunculus acer* L. in Germany. The larvae of both these species have been recorded from Northumberland by Bagnall and Harrison (1922). The second species is *P. orientalis* Felt reared in association with the gall midge *Kamptodiplosis reducta* Felt from leaf galls on *Siphonodon celastrineus* Griff. in the Philippine Islands. Felt (1918) tentatively placed it in this genus.

The species under consideration can be easily distinguished from either of the above species by the colour of its legs which in *P. ranunculi* are black and white and in the case of *P. orientalis* yellow to straw. There are various other differences. It has been decided to describe this species under the name *P. dizygomyzae* sp.n. on account of its larval habit.

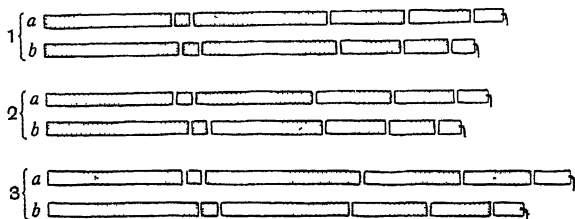
(a) *Description.*

Male. Length about 2 mm. Antennae: 2 + 12, proximal basal segment small, distal basal segment roundly quadrate; 1st and 2nd flagellar segments fused; basal enlargement on two proximal flagellar segments globular, on more distal segments subglobular, each such enlargement with ring of stout setae and a single whorl of regular circumfila; distal enlargement elongated, each bearing two whorls of regular circumfila and one ring of stout setae just proximal to distal circumfila; stem of 3rd flagellar segment about $1\frac{3}{4}$ –2 times as long as broad, neck about $2\frac{3}{4}$ –3 times as long as broad; stem of distal segment about $4\frac{1}{2}$ times as long as broad, distal enlargement about 4 times as long as broad. Palpi: each segment with short setae; proximal segment rectangular, the length half as long again as width; 2nd segment just over twice as long as first and slightly over 3 times as long as broad; 3rd segment about 3 times as long as and slightly narrower than first, about $4\frac{1}{2}$ times as long as broad; distal segment nearly 4 times as long as and slightly

narrower than first, about 7 times as long as broad. Wings: mottled yellow suffused with black, 3rd vein reaching margin just beyond tip of wing, costa interrupted at this point. Legs: distinctive, yellow and black, caused by the different colours of the hairs, proportions of black and yellow on tibia and tarsus of fore-, mid- and hind-legs as in female; claws curved at right angles, those of fore-legs bifid, those of others simple, empodium small. Genitalia: basal clasp segment stout with slight lobe; distal clasp segment moderately narrow; dorsal lamella deeply bilobed, each lobe rounded; ventral lamella about as long as or slightly longer than dorsal lamella, broad and roundly emarginate.

Co-types, Cecid. 1556, 1558, 1618, 1619, 1914-16 inclusive.

Female. Length about $2\frac{1}{2}$ mm. Antennae: 2 + 12, basal segments as in male; 1st and 2nd flagellar segments fused, 3rd flagellar segment about 3 times as long as broad, neck slightly longer than broad; distal



Text-fig. 11. Diagram of legs of: *a*, *Profeltiella dizygomyzae* sp.n.; *b*, *Profeltiella vespicoloris* sp.n. to show different proportions of black and yellow coloration. 1, fore-leg; 2, mid-leg; 3, hind-leg.

segment about $3\frac{1}{2}$ times as long as broad, distal enlargement about 3 times as long as broad, circumfila applied. Palpi: about as in male. Wings: deeper coloration than in male. Legs: black and yellow, proportions of black and yellow on tibia and tarsal segments of fore-, mid- and hind-legs as in Text-fig. 11 *a*. Ovipositor: lamelliform, very extensile, nearly as long as abdomen. Otherwise about as in male.

Co-types Cecid. 1557, 1909-13 inclusive.

Pupae, Cecid. 1669.

Larvae; gregarious, red, recognisable as *Profeltiella* sp. by the anal segment (Text-fig. 12).

Cecid. 1501-1503 inclusive.

Habitat. Larvae live in mines of *Dizygomyza barnesi* Hendel on *Salix* spp.

Another species of *Profeltiella*, of which a single female has been reared, is very similar to *P. dizygomyzae* but must be considered a distinct

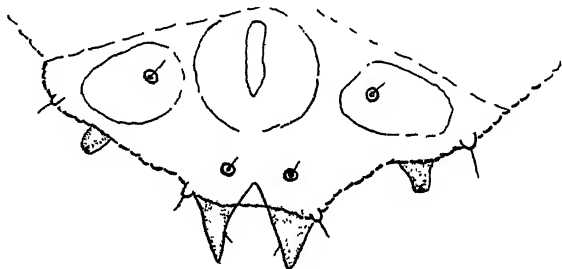
516 *A Cambium Miner of Basket Willows (Agromyzidae)*

species. It is proposed to name this species *P. vespicoloris* sp.n. and it can be described as follows:

Female. Very similar to female of *P. dizygomyzae*. May be separated from this species by the proportions of black and yellow on tibia and tarsal segments of fore-, mid- and hind-legs as shown in Text-fig. 11 *b*, in particular the 2nd tarsal segments are more yellow than in *P. dizygomyzae*. Palpi: very similar proportions to those of above species.

Type, Cecid. 1473.

Habitat. Single example reared from pot of soil in which *Dasyneura arabis* Barnes was being reared on *Arabis albidus* at Rothamsted, June, 1930, 3 months previous to the first finding of *P. dizygomyzae* larvae.



Text-fig. 12. Anal segment of larva of *P. dizygomyzae* sp.n. (diagrammatic).

(b) *Distribution.*

This midge seems to be widely distributed wherever the cambium miner occurs and is definitely associated with it. It has been reared from material collected in Lancashire and Hertfordshire, while larvae were found in the material sent from Suffolk.

(c) *Bionomics.*

The larvae live gregariously in the burrows of *D. barnesi* and can be found in large numbers in the larger cavities caused by rot setting in after the primary fly damage. Such cavities are doubtless partially due to the presence of these larvae in a burrow as well as to rot. The larvae are bright salmon pink in colour and may be recognised by the anal processes. When they are full grown, in August and September, they separate to some extent and spin white cocoons, still remaining in the burrows and cavities. Some viable larvae and pupae can, however, be found in the soil around the stubs, probably having fallen accidentally out of the rods. Pupation takes place in the spring, 8 days or so before emergence, although the cocoons are spun in the autumn. Plate XXX,

fig. 4, shows two cocoons, one at the terminal end of a tunnel and the other in a cavity.

Just before emergence of the adult the pupa wriggles its way along the burrow or from the cavity to the surface by means of the stiff spines on its dorsal surface. Normally emergence takes place in July, but pupae kept indoors in the laboratory from September onwards emerged successfully but spasmodically between January 26th and May 27th.

There is only one brood a year. So far this midge has only been found on *Salix viminalis*.

(d) *Parasites.*

The following Scelionid parasite has been reared from *Profeltiella dizygomyzae* collected in Lancashire: *Ectadius craterus* Walk. The parasitism figures vary considerably from year to year; in 1931 about equal numbers of midge and parasite were reared, but in 1932 36 parasites were reared to two midges. The parasite can be made to emerge earlier than usual by keeping it indoors in the winter, thus resembling its host.

VIII. SUMMARY.

1. A brief résumé is given of the information available concerning Dipterous cambium miners (Agromyzidae).

2. The morphology of the egg, larva, pupa and adult of *Dizygomyza barnesi* sp.n. Hendel is described.

3. The bionomics of this fly receive detailed attention. There is only one brood a year and the larvae mine in the cambium of different species of *Salix*. Pupation takes place in the soil. While *S. viminalis* is attacked most commonly, *S. triandra*, *S. purpurea*, *S. viminalis* × *purpurea* and *S. triandra* × *viminalis* occasionally suffer. In addition the larvae can live on *S. caerulea*. The damage caused by the larvae is considerable. The Braconids, *Symphia ringens* Halid. and *S. hians* Nees, are recorded as primary parasites.

4. Two gall midges are described, the larvae of one living as inquiline in the burrows of the *Dizygomyza* larvae. The bionomics of this species are described.

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EXPLANATION OF PLATES XXIX AND XXX.

PLATE XXIX.

- Fig. 1. A cambium miner of basket willows *Dizygomyza barnesi* sp.n. Hendel. Adult ♀, with outstretched wings. × 8.5.
- Fig. 2. The same, with wings in resting position. × 10.
- Fig. 3. The same, to show wing venation. × 20.
- Fig. 4. *Symphia ringens* Halid., parasite of *Dizygomyza barnesi*. × 10.
- Fig. 5. *Symphia hians* Nees, parasite of *Dizygomyza barnesi*. × 10.

PLATE XXX.

- Fig. 1. Section of 4-5-year-old *Salix viminalis*, showing fleck marks due to the tunnelling of the larvae of the *Dizygomyza*. Slightly diagonal. Natural size.
- Fig. 2. Cross-section of 2-year-old rod of Long Skin variety of *S. viminalis*, showing fleck marks and cankerous rot setting in. Slightly reduced.



Fig. 1.

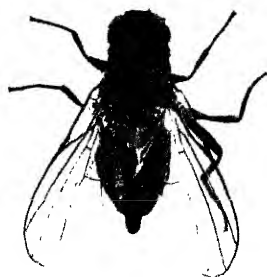


Fig. 2

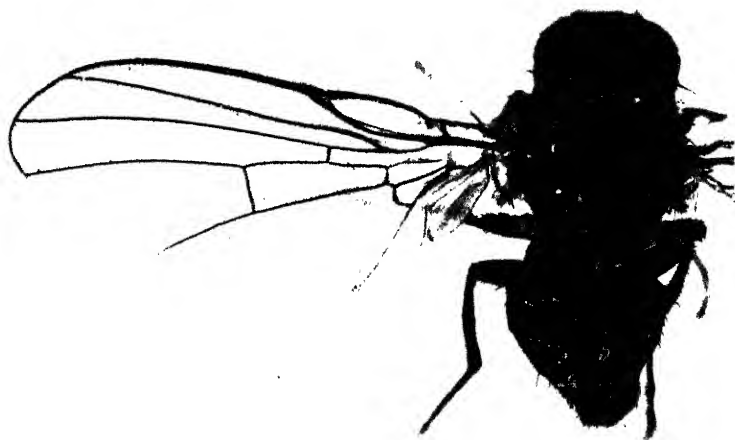


Fig. 3.



Fig. 4.



Fig. 5.

BARNES.—A CAMBIUM MINER OF BASKET WILLOWS (AGROMYZIDAE) AND ITS INQUILINE GALL MIDGE
PAR. CECIDOMYIDAE (pp. 498-519).



Fig. 1.



Fig. 2.

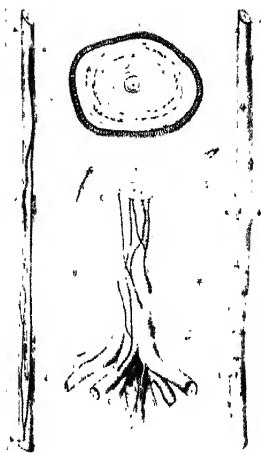


Fig. 3.



Fig. 4.



Fig. 5.



Fig. 6.

- Fig. 3. Reproduction of plate by Kienitz (1883). 1, cross-section of *Betula pubescens*, showing fleck marks. 2, peeled rod of *Salix rubra* Huds., showing tunnelling of the larva. 3, lower part of *Sorbus aucuparia*, showing fleck marks and tunnelling. 4, unpeeled rod of *S. rubra*, showing larva and exit hole. 5, dipterous larva. 6, head of larva.
- Fig. 4. Base of peeled Long Skin (*S. viminalis*) rod, showing two cocoons of *Profeltiella dizygomyzae* sp.n. in burrows of the *Dizygomyza*, a larva of which has been drawn in to indicate its appearance. Slightly enlarged.
- Fig. 5. Unpeeled rod of Long Skin (*S. viminalis*) showing cankerous appearance due to secondary attack by gall midge larvae and rot. Slightly reduced.
- Fig. 6. Same rod partially peeled, showing cavities in which the gall midge larvae congregate. Slightly reduced.

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REVIEWS

Cacao. By Dr C. J. J. VAN HALL. 2nd edition. Pp. xviii + 514. Macmillan and Co., Ltd., London, 1932. 28s. net.

Seventeen years have elapsed since the first edition of this book and, during this time, increase in knowledge and experience has rendered necessary many important modifications in our views regarding the cocoa plant and its culture. This has meant very extensive revision throughout the book and especially in those chapters dealing with cacao cultivation in the different countries and with diseases and pests. The new edition retains the general plan of the earlier book. Ch. I is a brief history of cacao during the last four centuries. Ch. II discusses the growth of cacao in its original home, tropical South America, and the geographical distribution of the cultivated plant especially in relation to temperature and rainfall. Ch. III is a rather tentative consideration of the scanty and unsatisfactory data relating to the chemical aspects of cacao soils, plant and produce. In Ch. IV, there is a key to the 18 described species of *Theobroma* followed by a detailed botanical account of the two cultivated species *T. cacao* L., and *T. pentagona* Bern., with notes on the non-cultivated species. Ch. V is a brief account of the varietal classification of cacao which is based almost entirely on seed and fruit characters. Ch. VI, which comprises 90 pages, is a very complete account of cacao cultivation beginning with forest clearing and proceeding through all stages of plantation establishment and welfare to fruit picking and replanting. Ch. VII outlines what little is known of the selection and breeding of cacao. Ch. VIII is a succinct account of the technical processes involved in the fermentation, washing and drying of the cacao seeds and their preparation for export. Ch. IX, comprising 80 pages, is an excellent digest of the diseases and pests of cacao, both in the plantation and in the storage shed, with brief measures for their practical treatment. Ch. X, comprising 192 pages, is the longest chapter in the book and is devoted to a consideration of the cacao-growing countries throughout the world, information being given about each country as regards development and statistics of the industry, suitability of climate and soil, export facilities, plantation and labour systems, methods of culture and preparation, yield and costings, diseases and pests. The last chapter, XI, deals with characteristics of the marketable bean in relation to market prices. There is a good Index, the book is illustrated by 176 text-figures and many tables, and numerous references to the literature of the subject are given as footnotes. The book is well produced and free from errors and misprints.

The volume is the only authoritative treatise in English dealing with cacao from all aspects and this second edition still further enhances the well-deserved reputation of its distinguished author. It is also a tribute to the very fine work carried out by Dutch scientists in the East Indies.

W. B. BRIERLEY.

Chromosomes and Plant-breeding. By C. D. DARLINGTON. Pp. xiii + 112. Macmillan and Co., Ltd., London, 1932. 7s. 6d. net.

This book is based on a series of articles that appeared in the *Gardener's Chronicle* during 1931, but additions have been inserted to make the text more useful to botanists and to give it the character of a general introduction to plant karyology. It is a fairly elementary exposition of recent developments in the study of plant chromosomes in relation to the practice of plant breeding and its usefulness to botanists lies in the fact that botanical examples are given of many phenomena more usually illustrated by animal instances. Indeed the name *Drosophila* only appears once in the book

and then quite casually. The first seven chapters briefly outline normal cytological processes (including a certain amount of controversial matter regarding meiosis), and the remaining ten chapters deal with ploidy in relation to new forms. Many of the chapters are absurdly short but they are packed with examples, and the book, small though it be, contains an astonishing amount of information. It is illustrated by 25 text-figures and a number of exceedingly useful tables. The use of the common name "hawkweed" for both *Crepis* and *Hieracium* may lead to a little confusion; Fig. 8 on p. 8 and Fig. 10 on p. 40 both refer to Text-fig. 9; Fig. 12 on p. 45 refers to Text-fig. 11; and there are misprints, one rather serious, on p. 89. The book contains a Foreword by Sir Daniel Hall and closes with a good Index. It will be useful to students who have no special knowledge of genetics and may find a wide circulation among keen growers who wish to know something of the rationale underlying the phenomena they meet with in daily practice.

W. B. BRIERLEY.

Recent Advances in Agricultural Plant Breeding. By H. HUNTER and H. MARTIN LEAKE, with a Foreword by Sir ROWLAND H. BIFFEN. Pp. x + 361, 16 Plates. J. and A. Churchill, London, 1933. 15s. net.

The aim of the book is stated to be "to present in a connected form the results of only such investigations as have advanced beyond the limits of purely academic interest and the outcome of which has been the introduction of improved varieties into general agricultural use." Following Sir Rowland Biffen's foreword and a general introductory chapter the book is divided into two parts, dealing respectively with crops of the temperate and tropical regions. Part 1 contains eight chapters dealing with wheat, barley, oats, flax, potato, forage-grasses, clovers and roots. Part 2 contains nine chapters classified according to the nature of the plant product-beverages such as coffee, cocoa and tea; sugar-cane; fruits such as citrus and banana; tobacco; opium-poppy; cereals such as maize, rice and sorghum; rubber; fibres such as cotton, sisal, flax and jute; oil plants such as coconut, oil palm, castor, ground-nut, sesame and soy bean. The book closes with full subject and author indices and, at the end of each chapter, are numerous references. There are 16 photographic plates.

Parts of the book give the impression of having been prepared in haste. Thus on p. 20 occurs the following: "Not the least remarkable feature of Yeoman is that, unlike the generality of hard wheats which are spring forms with a short period of growth, it is a winter variety under temperate climatic conditions, with the concomitant attribute of a long period of growth and, like most such varieties, is relatively prolific." On p. 23 the authors write: "...not the least remarkable feature of Yeoman is that, unlike most, if not all, the hard wheats which are spring forms with a short period of growth, it is a winter variety under temperate conditions with the concomitant attribute of a long period of growth and, like all such varieties, is relatively prolific." On p. 27 it is stated that the "existence of physiological forms (of rust of wheat) was established by Stakman and Piemeisel in 1917," a claim which these two workers would be the last to make. On p. 32 the authors first state that Wilhelmina wheat was produced by crossing Squarehead's Master with Zeeuwsche, and then that it was produced by crossing Squarehead with Zeeuwsche. Squarehead and Squarehead's Master are, of course, two very different wheats.

Apart, however, from such too frequent matters as these, which can be amended in a second edition, the book is a useful and suggestive compilation synthesising in small compass a very great number of scattered data many of which are not otherwise easily accessible. It is a little difficult to see for whom the book has been written. It is too much of a catalogue for the student and far too incomplete to be really useful to the research worker.

Two main impressions are left in one's mind after reading the book. One is that in spite of the comparatively short time during which the scientific breeding of agricultural plants has been carried on and the difficulty and complexity of perhaps most of the problems involved in such work, unexpectedly good progress has already been made with many important crops as regards not only yield but quality, resistance to diseases and pests, and the synthesising of new breeds for cultivation in localities where special climatic and other difficulties exist. The second is the extent to which further advance is becoming increasingly dependent on the correct application of the results of investigations outside the range of what may be regarded as pure plant-breeding. The achievement, however, is already great and, perhaps still greater, is the promise.

W. B. BRIERLEY.

Recent Advances in Botany. By E. C. BARTON-WRIGHT. Pp. 287, with 60 illustrations. J. and A. Churchill, London, 1932. 12s. 6d. net.

This book has rather the nature of a makeshift, attempting to cover certain regions of botany left by other volumes in the series, and its title gives little indication of its contents. Chapter I deals with "Some Theories regarding Plant Structure," and summarises investigations during the last decade on size and form in plants, the phyllode theory of the monocotyledonous leaf, the cotyledon and carpel polymorphism. The quality of this essay may be gauged by the fact that only the following authors are referred to, Adamson, Arber, Bower, Goebel, Parkin, Saunders and Wardlaw. Chapter II on "Palaeobotany" is a little better. Chapter III deals with "Experimental Methods in Relation to the Species Problem," and is largely a summary of Turessen's work. Chapter IV summarises 21 papers on the cytology of fungus reproduction and refers to three books. Chapter V summarises 44 papers on heterothallism, is rather badly written and contains numerous misprints. Students will not, from these two chapters, obtain much idea of what has really been interesting mycologists during the last ten years. Chapter VI summarises the work of sixteen authors on mycorrhiza. It is interesting to learn that the investigations of Bernard "finished at his death." Chapters VII and VIII deal with the Algae in a fairly adequate manner. Chapter IX summarises the present position on Virus Diseases of Plants, and although containing much at which to cavil, is, perhaps, the best chapter in the book. There are author and subject indices and 60 illustrations and the whole book is rather marred by misprints and unnecessary statements. Everyone realises the difficulties confronting an author in selecting the material for such a book as this and, although one admires Mr Barton Wright's courage, one cannot help feeling that the book is inadequate.

W. B. BRIERLEY.

Atlas der Krankheiten der landwirtschaftlichen Kulturpflanzen. Mit ausführlichen Textheften. Dritte Reihe: 11 Tafeln. In Mappe. Verlag von Paul Parey, Berlin, 1933. RM 10.

The third series of this well-known album of coloured reproductions of plant diseases and pests from paintings by A. Dressel is edited by Prof. Dr Otto Appel of Berlin-Dahlem and maintains, in general, the standard set by the preceding two series. The illustrations are as follow: Plate 1, Various *Fusarium* diseases of cereals; Plate 2, Leaf-stripe (*Helminthosporium gramineum*) and Net-blotch (*H. teres*) of barley; Plate 3, Twist of wheat (*Dilophospora graminis*); Plate 4, Corn Sawfly (*Cephus pygmaeus*) and Whiteheads of wheat (*Ophiobolus graminis*); Plate 5, Wheat Gout Fly (*Chlorops pumilionis*); Plate 6, Tubers and flowers of 15 varieties of potatoes resistant to Wart Disease (*Synchytrium endobioticum*); Plate 7, Eelworm (*Heterodera schachtii*)

on wheat and sugar-beet; Plate 8, Various diseases of grasses (*Claviceps purpurea*, *Fusarium* sp., *Sclerotium rhizodes*, *Epichloë typhina*); Plate 9, Dodder (*Cuscuta racemosa*, *C. trifolii* and *C. epilinum*) on clovers and flax; Plate 10, Various tomato diseases (*Phytophthora infestans*, *Fusarium acuminatum*, *Phoma destructiva*, *Macrosporium tomato*, *Botrytis cinerea*); Plate 11, Downy mildew of hop (*Pseudoperonospora humuli*). In some of the plates the green colours are a little strident in tone, but, on the whole, the colour reproduction is exceedingly well done, and certain of the plates, e.g. that showing tomato diseases, are really beautiful. Although plate numbers have been used above, there is actually no serial numbering of the plates, which is decidedly inconvenient. Accompanying the plates is a useful pamphlet of 27 pages describing the various diseases illustrated and briefly indicating methods of control. The reproductions are 31 by 45 cm., printed on thin but good quality paper, and are very suitable for framing and placing round the walls of classrooms and laboratories at eye level.

W. B. BRIERLEY.

The Farm and the Nation. By Sir JOHN RUSSELL. Pp. 240. George Allen and Unwin, Ltd., London, 1933. 7s. 6d. net.

It would need a fairly long essay to review this book in any adequate sense so tightly is it packed with knowledge and the wisdom of great experience. There can be but few people who have, at first hand, seen so much of the largenesses and the detail of agricultural conditions and processes in so many different lands as Sir John Russell, and perhaps fewer still who can put their knowledge and experience into such simple readable language. Indeed so easily—almost slickly—is the book written that occasionally one wonders whether the present-day situations may not be a little more obscure than is conveyed by the author and whether their explanation both historically and economically may not be a little more difficult. Much of the material of the book has already been presented in wireless talks and popular lectures, and under such conditions a tendency to over-simplification for the sake of clarity and brevity is difficult to resist. There is also in the book a certain amount of repetition and awkward phraseology, and occasionally the writing borders on the naïve. After an introductory chapter on the sources of the nation's food the author devotes three chapters to British farming followed by two chapters on Empire farming. The next three chapters dealing with the farmer's difficulties, the better usage of land, and the land absorption of displaced men, are followed by a brief summary chapter, a valuable appendix on sources of information and an adequate index. The book is illustrated by numerous tables and very clear graphs. The book is one to be read by all interested in agriculture and it could very usefully find its way into the hands of students of pure science. One has the feeling that if the author had taken the book slightly more seriously and given more time and thought to its production he could have made of it a little masterpiece which would stand as one of the minor classics of English agricultural literature.

W. B. BRIERLEY.

The Medicinal and Poisonous Plants of Southern Africa. By J. M. WATT and M. G. BREYER-BRANDWIJK. Pp. xx + 314. E. and S. Livingstone, Edinburgh, 1932. 25s. net.

The purpose of this book is "to give all the available information on the medicinal uses, chemical composition, pharmacological effects, and human and veterinary toxicology of the flora of Southern Africa." The plants are arranged according to the system of Harms taken from Phillips' *The Genera of South African Plants*, and all the specimens dealt with by the authors were identified by the Division of Plant Industry at Pretoria and are housed in the National Herbarium there. The main

portion of the book consists of 127 Sections of very varying length, each, save in a few cases, devoted to a single family. The genera of each family and the species of each genus are considered seriatim and at the end of each Section are very complete references in which unfortunately the titles of the papers are not cited. There are two Appendices the first being a list of 28 publications referred to in the text by the name of the author and the second a detailed schedule in both English and Afrikaans of a standard method for the reporting and sending of specimens. The book closes with four useful Indices: the first containing botanical names, the second English and Afrikaans names with their botanical equivalents, the third native names with their botanical equivalents and the fourth the names of active principles with the species from which they are derived. These Indices comprise 88 double-column pages and are unusually complete and well done. The volume is illustrated by twelve full-page plates in colour which are both accurate and beautiful, and fourteen black and white plates some of which are reproduced rather poorly from Government publications. No misprints or errors have been noted, the volume is unusually well printed and produced and as modern prices go is cheap.

The book brings together an enormous mass of information from medicine, chemistry, pharmacology and toxicology both in their modern scientific aspects and also in their native folk-lore and traditional aspects. So far as one can judge it is not only encyclopaedic as regards the literature of the subject but it also contains much original observation and recording by the authors and is written in a critical spirit throughout. The book is undoubtedly one of first class importance and lasting value, in many ways setting a standard in the subject, and it should find a place in every botanical library.

W. B. BRIERLEY.

STUDIES IN THE PHYSIOLOGY OF THE VIRUS DISEASES OF THE POTATO

II. A COMPARISON OF THE CARBOHYDRATE METABOLISM OF NORMAL WITH THAT OF CRINKLE POTATOES; TOGETHER WITH SOME OBSERVATIONS ON CARBOHYDRATE METABOLISM IN A "CARRIER" VARIETY

BY EUSTACE BARTON-WRIGHT, M.Sc.
AND ALAN M^cBAIN, B.Sc.

*(From the Scottish Society for Research in Plant Breeding,
Corstorphine, Edinburgh.)*

(With 12 Text-figures.)

I. INTRODUCTION.

It was shown by us in a previous communication⁽¹⁾ that potato plants affected with leaf-roll differed fundamentally in their carbohydrate metabolism from healthy plants, and that the sugar of transport, in the healthy potato is sucrose, whereas in the leaf-roll plant it is hexose. In the circumstances it was thought to be of sufficient interest and importance to extend our observations to other potato virus diseases, in the hope of throwing some light on the nature of these diseases and to ascertain in what way they differ in their effect on the host plant. We have at the same time investigated the effect of a latent virus (paracrinkle) in a "carrier" variety, in order to determine whether there is any fundamental difference in metabolism between healthy and apparently healthy plants.

The particular diseases to be described here are known as crinkle and paracrinkle. Crinkle has also been called crinkle "A" by Salaman⁽¹¹⁾, but this is a synonym. Paracrinkle is a disease first described by Salaman and Le Pelley⁽¹³⁾ as being carried by King Edward (see below).

Murphy⁽⁶⁾ was the first investigator to differentiate the disease group crinkle from other mosaic diseases to which the potato is prone. Quanj⁽¹⁰⁾, working in Holland at the same date, also described a disease in the variety Eigenheimer, which he claimed to be an intensified form of mosaic and which he termed "Welvingziekte." Both investigators, however, are now agreed that they were studying one and the same disease.

According to Murphy a potato plant affected with crinkle exhibits the following clinical picture:

"Affected plants are typically bushy, dwarfed specimens of about the same size and form as the low-headed type of leaf-roll. The compact appearance of curly dwarf is absent. The colour is a pale green, but this feature is not marked. The most characteristic symptom is a pronounced and characteristic puckering and downward curling of the leaves. There is no distinct spotting as in mosaic, but diffused, slightly yellowish areas occur all over the foliage. As death approaches this colour becomes more pronounced and is accompanied by rusty, brown spots beginning near the tips of the leaves. The foliage is brittle and easily injured. There does not appear to be in normal crinkle any discoloration of the vascular tissues of the leaf or stem, such as occurs in streak (Orton⁽⁹⁾). The feature is present sometimes, but it is believed to be a complication. The plants usually live until the end of the season, behaving in this respect more like leaf-roll or mosaic than curly dwarf."

The description given of the disease by Quanjer does not materially differ from that of Murphy. The American workers Schultz and Folsom^(15, 16), as well as Valleau and Johnson⁽¹⁹⁾ and Koch⁽⁵⁾ considered that a disease described originally by Schultz and Folsom as "Medium plus Mosaic," and which these two workers later considered to be identical with a disease known as "Rugose Mosaic"⁽¹⁵⁾, are both identical with Murphy's crinkle. Murphy and McKay⁽⁸⁾, however, from a comparison of American potato virus diseases with European types, consider such identity to be improbable.

Paracrinkle is a disease present in a latent condition in King Edward. It was shown by Salaman and Le Pelley that, when scions from robust and apparently healthy King Edward plants were grafted on Arran Victory, a severe crinkle-like disease made its appearance. On the other hand, when King Edward was grafted on President, no sign of disease made its appearance (occasionally there was a fleeting mottle), but nevertheless the disease was present in the President plants, for when scions were removed from these and grafted back to Arran Victory, the disease made its appearance. We have been able to confirm this work completely. King Edward thus appears to be a perfect carrier.

The clinical symptoms of paracrinkle in Arran Victory develop very irregularly and usually commence with the appearance of yellow-white blotches or spots, and this is followed rapidly by waving and deformity of the leaves. Clearing of the veins is not so prominent nor so constantly an early feature of the disease as in ordinary crinkle. Plants grown in the

subsequent season show stems which are short, numerous and brittle; and the foliage is clumped and grossly deformed.

At the present time the literature of the mosaic group of virus diseases of the potato is passing through a phase of extreme confusion, owing to the fact that diseases which at one time were thought to be due to single entities have now been shown to be composite in character. For example, it has been demonstrated by Kenneth Smith⁽¹⁷⁾ that certain diseases of the mosaic group can be separated into two components, which he has termed *X* and *Y*. Juice inoculation or grafting introduces both elements, *i.e.* *X* and *Y*, whereas the insect vector, *Myzus persicae*, is selective in its action and only transmits the *Y* portion of the complex. Salaman⁽¹²⁾ has now gone further and claims that there is a third element present, *Z*, and that this *Z* virus is a component of crinkle and paracrinkle. Thus, according to Salaman, crinkle has the composition *XYZ*, whereas paracrinkle has the composition *ZY'*. The *Y* part of the complex in paracrinkle is considered for a variety of reasons to differ from the ordinary *Y* of mosaic and crinkle, and hence it is symbolised as *Y'*. The evidence brought forward by Salaman to demonstrate the existence of *Z* is at present meagre and depends entirely on the validity and interpretation of one experiment.

In a recent communication Murphy and McKay⁽⁷⁾ have also shown that crinkle is a compound disease and they have been able to synthesise it in a convincing manner. Thus they found that the variety Irish Chieftain is a carrier plant and that apparently healthy Irish Chieftain contains a latent virus which they call virus *A*. They showed that when simple mosaic was introduced into plants of Irish Chieftain, symptoms indistinguishable from crinkle were produced, and that these symptoms persisted for at least two or three seasons. Further, when this crinkle-like disease was transferred to President, typical crinkle symptoms were produced in the majority of cases.

For the physiological work to be described in this communication, the standard crinkle described by Murphy was used. We wish to express our thanks to Prof. Murphy for a supply of tubers of Irish Chieftain infected with crinkle which served as our source of the disease.

So far as we are aware, no systematic investigation of the carbohydrates of crinkle infected plants has been made heretofore, and the present work was initiated and carried out on the same lines as was employed by us for leaf-roll. It should also be mentioned that our colleague, Mr George Cockerham, has carried out a similar investigation on mild mosaic at the sub-station, Gibston, Huntly, Aberdeenshire.

II. METHODS.

(1) *Material.* The variety Arran Victory was employed throughout this investigation for the experimental work on crinkle, while President was used for paracrinkle. The source of crinkle was tubers of Irish Chieftain obtained from Prof. Murphy, and our source of paracrinkle was from stock seed of King Edward supplied to us by Mr Adam Millar, B.Sc., of the Seed Testing Station, East Craigs, Corstorphine, Edinburgh. We are also indebted to Dr R. N. Salaman for kindly giving us some tubers of President carrying paracrinkle, with which we were able to compare our own material.

(2) *Transmission of the diseases.* Both diseases were transmitted by stem-grafting with the appropriate scions, Irish Chieftain for crinkle and King Edward for paracrinkle. We originally proposed to carry out a similar series of experiments on paracrinkle in Arran Victory as was done for crinkle, but for a variety of reasons this programme was abandoned. In order to obtain material of paracrinkle in Arran Victory for this proposed work we grafted King Edward to this variety, and in 57 cases out of 60 the disease made its appearance in the same way and with the same characteristic symptoms as described by Salaman and Le Pelley. In no case did our King Edward plants from which the scions were taken show the faintest trace of mottle and all grew to be robust and apparently healthy plants. A curious feature was discovered in this connection. We originally attempted to transmit paracrinkle to Arran Victory by Murphy's method of core-grafting with King Edward. In no case did the disease make its appearance. The plants were allowed to grow undisturbed for the whole season and remained to all outward appearance perfectly healthy and showed no sign of mottle, but they died down some three weeks earlier than the controls. This work was repeated this season (1932) with exactly the same result. Moreover, last season's tubers grown on this year still maintained this feature of earlier ripening. On the other hand, core-grafting of King Edward to President produced no effect and grafted and control plants died down at the same time, nor did this core-grafting to an early variety (Arran Crest) produce any earlier ripening. It is evident that the King Edward core transmits some peculiarity to Arran Victory to cause this result.

(3) *Duration of experiments.* The duration of our experiments with crinkle was the same as in our work on leaf-roll, namely nineteen hours, and the samples were gathered at hourly intervals. In the case of paracrinkle, the experimental period was extended to twenty-four hours, and

since we were merely concerned in seeing whether there were any significant differences between healthy and apparently healthy plants, samples were gathered at two-hourly intervals.

(4) *Preparation of material.* The material was killed and worked up in the same way as in our other communication. The clearing of the solutions was effected, however, with colloidal ferric hydroxide and not lead acetate (see in this connection Thomas(18) and Evans(3)).

(5) *Estimation of carbohydrates.* The Schaffer-Hartman micro-method (14) of estimating sugars was used throughout this work. We found this to be a very convenient, rapid and accurate method of estimating sugars and as many as 120-150 estimations can be carried out in a day.

Acid hydrolysis of the sugar solutions was abandoned for sucrose determinations and a preparation of invertase from Messrs British Drug Houses was used instead. Starch hydrolysis was carried out with ptyalin as before.

(6) *Expression of results and statistical treatment.* The results are again expressed as a percentage of the "Residual Dry Weight" and the necessary correlation coefficients, regressions and significant differences have been calculated. We have again employed the method of *t* instead of calculating the probable error (see Fisher(4)).

(7) *Grading.* The grading of our plants was carried out in the same way as for our leaf-roll work and sixty plants of healthy and sixty of diseased were used for each experimental run. The individual samples of laminae and petioles made at the hourly collections were composed of 50 gm. fresh weight. All plants were grown in insect-proof greenhouses.

III. EXPERIMENTAL OBSERVATIONS.

The first experimental run was carried out on President healthy and President carrying paracrinkle on May 16th, 1932, and the first run on crinkle in Arran Victory on May 23rd, 1932. The second runs on President and Arran Victory were carried out simultaneously on June 27th, 1932.

A. CARBOHYDRATE FLUCTUATIONS IN HEALTHY AND CRINKLE POTATOES.

Arran Victory, Series I (May 23rd, 1932).

(1) *Moisture changes.* The moisture changes in healthy laminae and petioles are shown in Fig. 1 and the corresponding values for crinkle plants in Fig. 2. The results have again been calculated as the weight of water per 100 gm. residual dry weight. In the case of the healthy plants

the mean value for the laminae over the experimental period of nineteen hours was 834.4 gm. and for the petioles 2213 gm., while for the crinkle plants the corresponding values were laminae 791 gm. and petioles

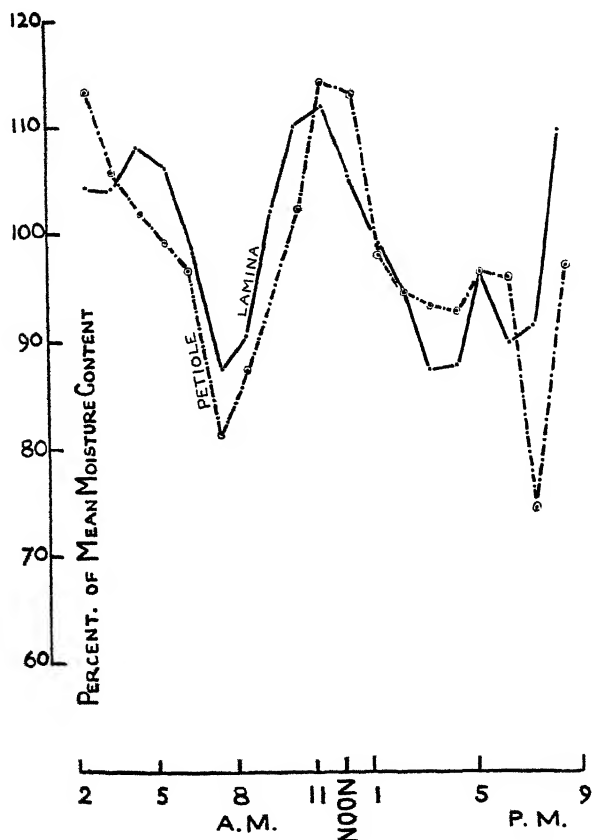


Fig. 1. Variations in the moisture content of laminae and petioles of healthy Arran Victory plants (Arran Victory, Series I). Moisture content calculated as grams moisture per 100 gm. residual dry weight and shown as the percentages of the mean for laminae and petioles. The points that have been plotted are the means of successive pairs of observations.

2104 gm. It will be seen that although the water content of the diseased material is lower than the healthy it does not show the great differences found for leaf-roll. Similarly, the curves for both diseased and healthy follow a similar trend and show maxima and minima at the same times.

(2) *Carbohydrate fluctuations in the laminae of healthy and crinkle material.* The variations in hexose, sucrose and starch in the healthy leaf-blade are shown in Fig. 3 and the corresponding fluctuations in crinkle plants in Fig. 4. It is clear from the graphs that the trend of carbohydrate

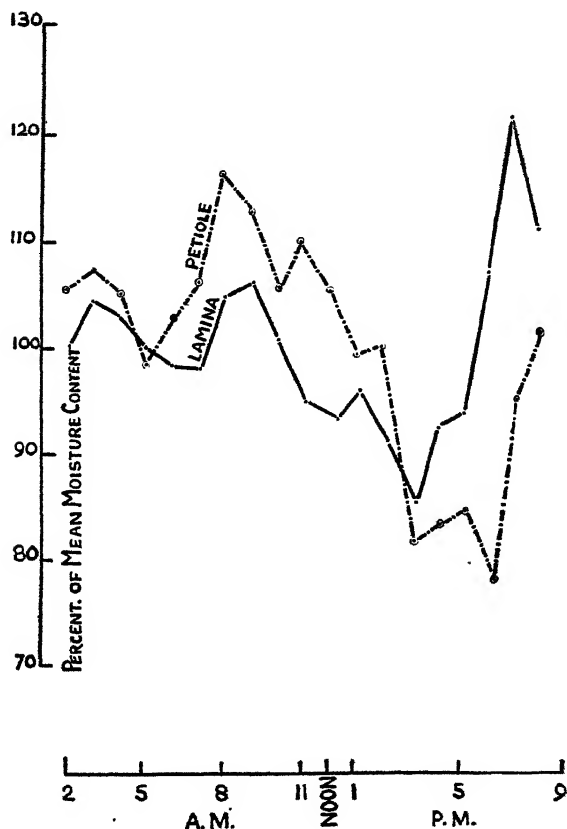


Fig. 2. Variations in the moisture content of laminae and petioles of crinkle plants (Arran Victory, Series I). Moisture content calculated as in healthy material and means of successive pairs of observations plotted in the curves.

variation over the experimental period is similar in the two cases. The two main differences to be noted are: (1) that the percentage values (for the total experimental period) for hexose, sucrose and starch are higher in the crinkle laminae than in the healthy, and (2) the fluctuations in the healthy leaf-blade tend to lag a period of two hours behind those in the crinkle laminae.

That there is no statistical significance to be attached to carbohydrate changes in the two cases is further shown when the significance of

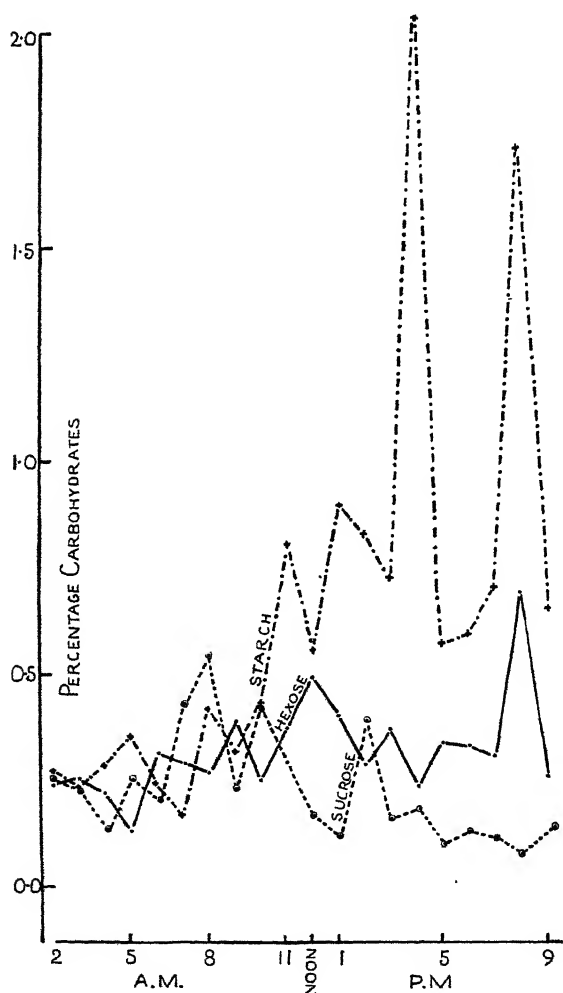


Fig. 3. Variations in hexose, sucrose and starch in the laminae of healthy Arran Victory plants (Arran Victory, Series I).

the differences of the means is calculated. In Table I we give the values of t for the differences of means of hexose, sucrose and starch calculated by the elaborated "Student's" method described by Fisher (4).

Table I.

Significance of differences of means of values for hexose, sucrose and starch in healthy and crinkle laminae.

	$S(x - \bar{x})$	t	P
Hexose	0.0787	1.2720	0.2-0.1 Not significant
Sucrose	0.1009	0.9767	0.4-0.3 "
Starch	0.0185	0.1227	> 0.9 "

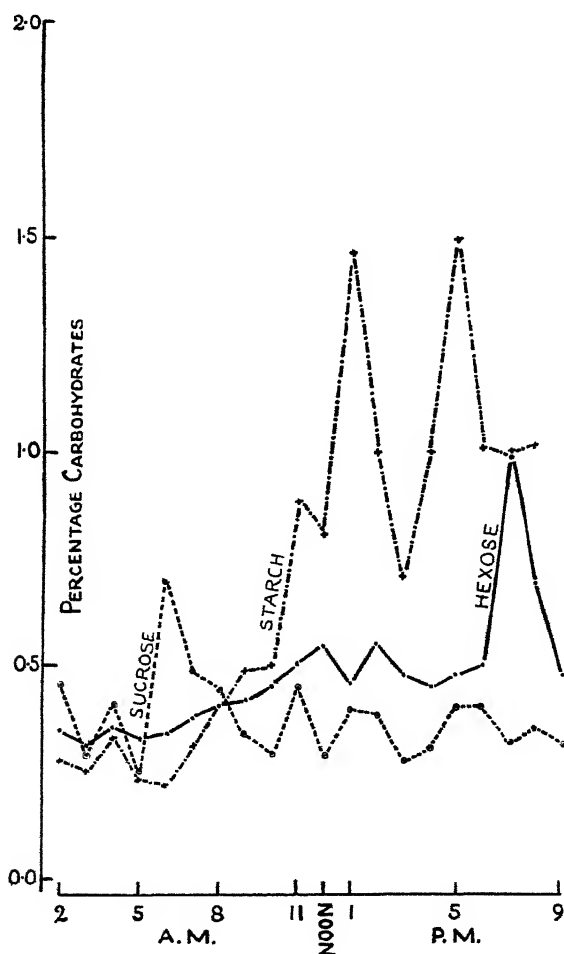


Fig. 4. Variations in hexose, sucrose and starch in the laminae of crinkle plants (Arran Victory, Series I).

It is thus evident that in the early stages of crinkle the formation of carbohydrates proceeds on the same lines as in healthy plants and does not show the marked differences found for leaf-roll. On the other hand, the curves for the diseased material do show that the percentage carbohydrates are higher than in the healthy and that the fluctuations in the latter tend to lag behind the crinkle, and the question arises as to the significance to be attached to these two features. Another point that should be noticed is that the general trend of the curves of the sucrose values is, on the whole, lower than the hexose and brings this series of observations into line with our experimental results in Arran Victory, Series II, in our leaf-roll paper. We were unfortunately precluded from making any estimations at a very early stage of growth owing to our method of transmitting the disease by stem-grafting.

B. SUGAR FLUCTUATIONS IN THE PETIOLES OF HEALTHY AND CRINKLE MATERIAL.

We have already shown that sucrose is the sugar of translocation in the healthy potato plant, whereas in leaf-roll plants once the lamina is left no trace of sucrose is to be found till the tubers are reached and the only sugar in petiole and stem is hexose, and presumably therefore the sugar of translocation in leaf-roll plants is hexose. In the circumstances it became necessary to determine whether a similar situation was in existence for crinkle plants. This, however, was not found to be the case. Sucrose was abundant in the diseased petioles. In Fig. 5 we have plotted the sucrose of healthy and diseased petioles, and to show the drift with time with greater clarity, the means of successive pairs of observations have been plotted. It will be seen that in the early part of the day sucrose is abundant in the crinkle petioles, and rises to a maximum at 10 a.m. and a second maximum is shown at the close of the experimental period. In the healthy petioles, the sucrose values remain relatively constant for the first seven hours and then abruptly rise to their first maximum at 11 a.m., fall and rise to a second maximum at 7 p.m., and a final high maximum is attained at the end of the experimental period. When the significance of the differences of means is calculated $S(x - \bar{x}) = 0.0587$, $t = 0.2834$, $P = 0.8-0.7$. Here, again, there is no significance between sucrose in healthy petiole and sucrose in diseased petiole, since P is not significant.

From these results it is clear that in its early stages crinkle does not have the marked effect on carbohydrate metabolism that leaf-roll has,

and it is of interest to compare these results with those obtained at a later period of the growing season.

Arran Victory, Series II (June 27th, 1932).

The summer of 1932 was one of bright sunshine and hot weather and, under the conditions of our insect-proof greenhouses with their thin wire gauze protection, it became a matter of considerable difficulty to prevent the temperature from reaching very high values. On this account the plants began to ripen off early, and at the time our second experimental

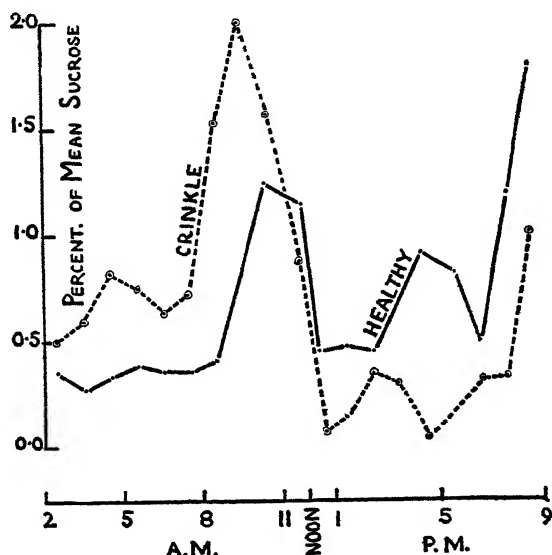


Fig. 5. Petiolar sucrose in healthy and crinkle Arran Victory plants (Arran Victory, Series I). The means of successive pairs of observations have been plotted in the figures to show the drift with time with greater clarity.

run was made both healthy and diseased material was beginning to show marked signs of senescence.

(1) *Moisture changes.* In the healthy material the mean moisture value over the experimental time was for the laminae 806.3 gm. and for the petioles 1607 gm., while for the crinkle plants the corresponding values were laminae 795.6 gm. and petioles 1875 gm. Comparison of these values with those of Arran Victory, Series I, show that there has been a fall in the water content of the healthy laminae (806.3 as against 834.4 gm.) and a similar fall in the petiolar values (1607 as against 2213 gm.), whereas in the crinkle laminae there has been little change in

moisture content (795.6 as against 791 gm.), but there has been a fall in the petiolar values, although this is not so marked as in the healthy petioles (1875 as against 2104 gm.).

The curves for healthy and crinkle moisture changes are shown in Figs. 6 and 7. It will be seen that the shapes of the curves in both cases

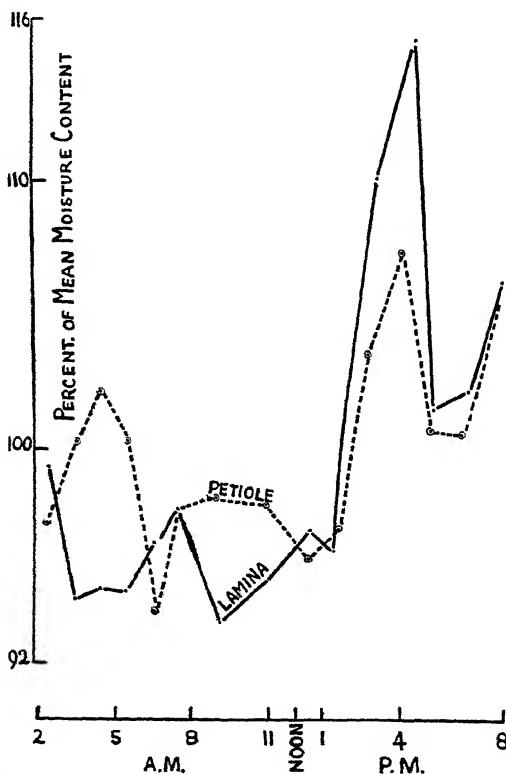


Fig. 6. Variations in moisture content of laminae and petioles of healthy Arran Victory plants (Arran Victory, Series II).

are different from those found for Arran Victory, Series I (Figs. 1 and 2). In the case of the healthy laminae and petioles, the curves follow one another closely, and in the laminae after attaining a minimum at 9 a.m., gradually rise to a maximum at 4 p.m. The petiolar curve, on the other hand, does not commence to rise until 1 p.m. In the curves for the diseased material, the minimum point for the laminae is reached at 1 p.m., four hours after the healthy, and thereafter rises to a maximum

at 7 p.m. It is, however, in the crinkle petiolar curve that the most curious fluctuations are to be observed. An unusually high maximum is attained at 11 a.m., maintained for one hour and then falls away abruptly

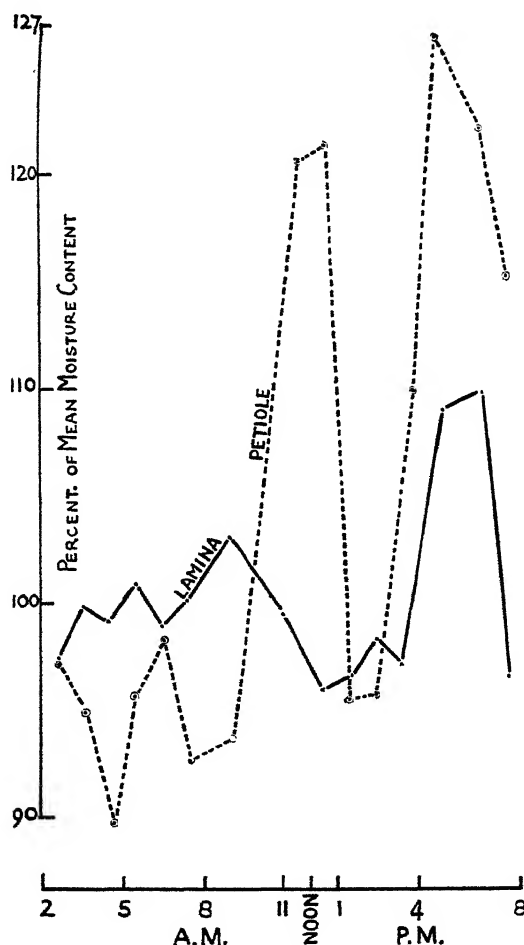


Fig. 7. Variations in moisture content of laminae and petioles of crinkle plants (Arran Victory, Series II).

to a minimum, thereafter petiolar and laminae curves fluctuate together. We are quite unable to account for this peculiar result. It is clear, however, that in the later stages of the disease water does not enter the lamina with the same facility as in the healthy plant. It will be recalled

that we found a similar state of affairs in leaf-roll plants and it is of interest to compare Fig. 7 in this case with Fig. 8 in our leaf-roll paper.

(2) *Carbohydrate fluctuations in the laminae of healthy and crinkle material.* The variations in hexose, sucrose and starch in the healthy laminae are shown in Fig. 8 and the corresponding values for the crinkle plants in Fig. 9.

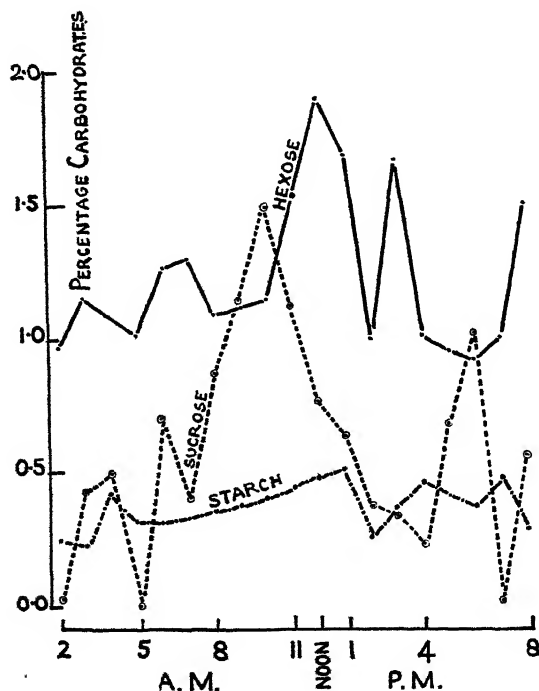


Fig. 8. Variations in hexose, sucrose and starch in the laminae of healthy Arran Victory plants (Arran Victory, Series II).

In the healthy laminae the hexose values are higher than the sucrose and the latter reach a first maximum at 10 a.m., and then fall away until 6 p.m., when a second maximum is attained. The starch values are very low throughout the entire period. In the case of the crinkle laminae, the sucrose percentages are higher than in the healthy, and although the first maximum is reached at the same time as in the healthy (10 a.m.), and the second occurs at 3 p.m., three hours earlier than the second maximum in the healthy plants. The starch values are again very low.

We have calculated the significance of the differences of means between healthy and diseased and the values are shown in Table II.

Table II.

	$S(x - \bar{x})$	t	P
Hexose	0.2090	2.2490	0.05-0.02 Significant
Sucrose	0.5629	2.8450	<0.01 "
Starch	0.0495	1.4460	0.2-0.1 Not significant

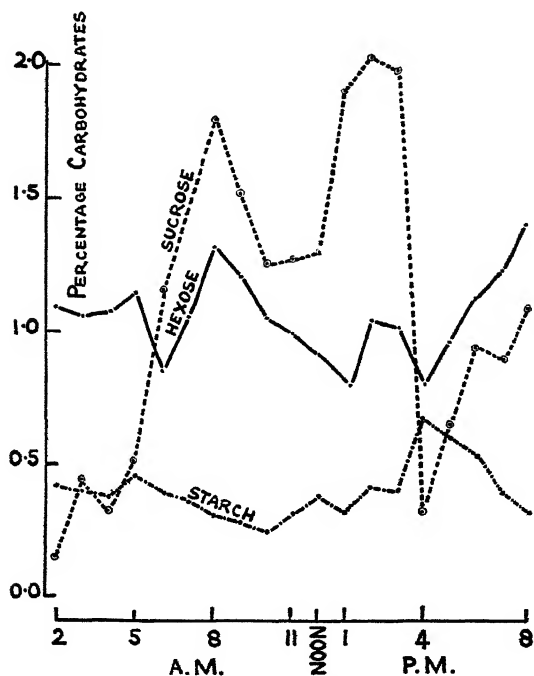


Fig. 9. Variations in hexose, sucrose and starch in the laminae of crinkle plants (Arran Victory, Series II).

In this instance, at a later time in the growing season, there is a significant difference between hexose healthy and hexose crinkle and also between sucrose healthy and sucrose crinkle, while there is no significant difference between starch values. We have already stated that at the particular period at which this second set of values was obtained the plants showed distinct signs of senescence and it is therefore of interest to compare the partial regressions in the two cases. Calling hexose X , sucrose Y , starch Z and temperature T , we have in the case of the healthy laminae the following equations:

(a) *Healthy laminae.*

$$X = 0.275 + 0.0746Y + 10.78Z + 0.0331T \dots(1),$$

$$Y = -1.415 + 1.564X + 0.6976Z - 0.0094T \dots(2),$$

$$Z = 0.0027 + 0.0767X + 0.0237Y + 0.0145T \dots(3).$$

It is clear from (1) that fluctuations in hexose are dependent on fluctuations in starch and that neither sucrose nor temperature play any significant part. Similarly from (2) sucrose formation is dependent upon both hexose and starch, but hexose has relatively the greater influence and temperature plays no significant part in the matter. Lastly, from (3) it will be observed that neither hexose, sucrose nor temperature have any significant effect on starch formation. In other words, starch formation is not taking place to any marked extent and the main reaction that is proceeding in the leaf-blade is the breakdown of starch to soluble sugars.

It is thus evident that at this stage in the life of the healthy plant when senescence is rapidly approaching, photosynthesis has either entirely ceased or its products are very small in amount, and that the formation of soluble sugars is brought about by the hydrolysis of reserve starch that has been previously built up in the leaf-blade and not by photosynthesis.

Turning now to the situation in the crinkle laminae we have the following regressions to consider:

(b) *Crinkle laminae.*

$$X = 2.658 + 0.0384Y - 1.943Z - 0.0473T \dots(1),$$

$$Y = -1.392 + 0.3166X - 3.822Z + 0.2162T \dots(2),$$

$$Z = 0.522 - 0.4774X - 0.1139Y + 0.0290T \dots(3).$$

Here, again, it is clear from (1) that hydrolysis of starch is the main factor concerned in the formation of hexose and that neither sucrose nor temperature play any significant part in the matter. From (2) hydrolysis of starch is the paramount reaction concerned in the formation of sucrose, and this reaction is of greater extent than formation of hexose from starch and, further, such influence as temperature has is also mainly concerned with the formation of sucrose, although it is not of great significance. Finally from (3), as with the healthy leaf-blade, neither hexose, sucrose nor temperature play any significant part in the formation of starch.

It would therefore appear from the nature of the regressions that in the crinkle laminae, as in the healthy, photosynthesis has practically ceased, with the difference, however, that the hydrolysis of starch is in the main leading to the formation of sucrose. It will be more convenient

at this stage to postpone this question of sucrose formation in healthy and diseased material to the discussion.

C. SUGAR FLUCTUATIONS IN THE PETIOLES OF HEALTHY AND CRINKLE MATERIAL.

To show with greater clarity the drift with time of sucrose in the petioles of healthy and diseased material, we have plotted in Fig. 10 the mean points of the sucrose percentages in the two cases.

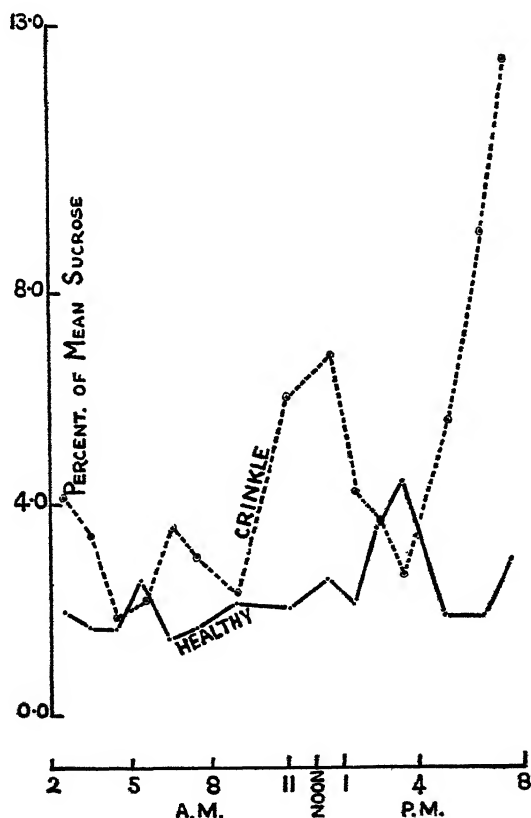


Fig. 10. Petiolar sucrose in healthy and crinkle Arran Victory plants (Arran Victory, Series II). The means of successive pairs of observations have been plotted.

Two facts emerge from a consideration of the curves: (1) the higher percentage of sucrose in the diseased petioles, and (2) the greater accumulation of sucrose in the crinkle petioles towards the close of the day. We

would interpret this result as showing that the sucrose cannot flow out of crinkle petioles with the same readiness as in healthy plants. In fact, from an inspection of the curves it would appear that the sucrose in the crinkle petioles is alternately dammed up and then released and that the channel of transport cannot cope with the supply from the source. It may be on this account that there is such a marked accumulation of sucrose in the leaf-blade of the diseased material at the close of the growing season. We have also calculated the significance of the differences of the means: $S(x - \bar{x}) = 2.765$; $t = 2.641$; $P < 0.01$. P is fully significant,

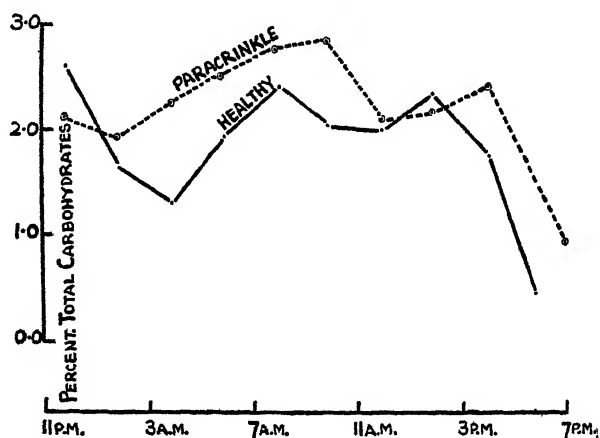


Fig. 11. Diurnal variations in total carbohydrates (hexose, sucrose and starch) in the laminae of healthy and paracrinkle carrying President plants (President, Series I). The means of successive pairs of observations have been plotted in the figures.

and it is thus evident that the behaviour of sucrose in the petioles of healthy and diseased plants is dissimilar at the close of the growing season.

D. CARBOHYDRATE FLUCTUATIONS IN THE LAMINAE OF HEALTHY PLANTS AND POTATOES CARRYING PARACRINKLE.

President, Series I (May 16th, 1932), and *President, Series II*
(June 27th, 1932).

For the sake of convenience we have considered it best to show the curves for total carbohydrates (*i.e.* hexose, sucrose and starch) in the two series of observations that were made between healthy and apparently healthy plants. It is evident from the curves (Figs. 11 and 12) that the

diurnal trend of carbohydrates is similar in both cases and that senescence has produced no marked change in the apparently healthy in comparison with the healthy. This is further borne out when a consideration is made of the significance of the differences of the means:

President, Series I.

$S(x - \bar{x}) = 0.3090$, $t = 1.1770$, $P = 0.3-0.2$. Not significant.

President, Series II.

$S(x - \bar{x}) = 0.3140$, $t = 0.9346$, $P = 0.4-0.3$. Not significant.

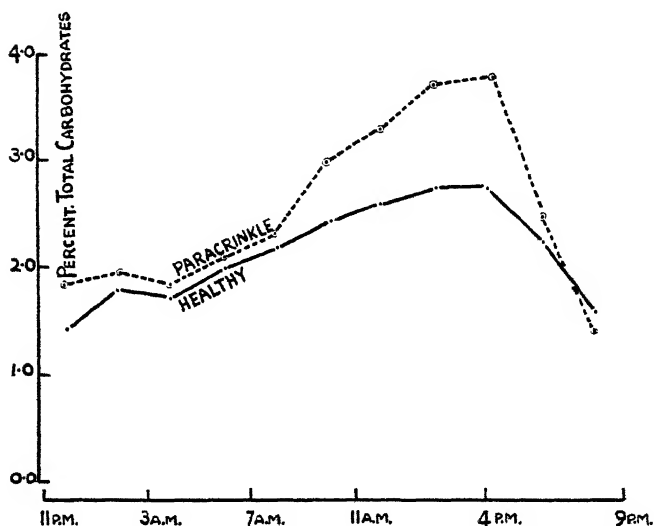


Fig. 12. Diurnal variations in total carbohydrates (hexose, sucrose and starch) in the laminae of healthy and paracrinkle carrying President plants (President, Series II). The means of successive pairs of observations have been plotted in the figures.

P in neither case is significant. The presence of a latent virus in a variety has therefore no significant influence upon the carbohydrate metabolism and the behaviour of the plants in this respect is similar to the healthy. Nevertheless, although the differences between healthy and apparently healthy is not statistically significant, the curves in both series of observations do show that the percentage of total carbohydrates in the paracrinkle plants is always slightly higher than in the healthy. It is difficult to say whether this slight difference might not become exaggerated in subsequent growing seasons. On the evidence at present available this would appear to be unlikely. For example, we have grown tubers of

President carrying paracrinkle supplied to us by Dr R. N. Salaman for two seasons, and the plants have shown no sign of lack of vigour, nor have we been able to detect the presence of any mottle on the leaves.

IV. DISCUSSION.

The data we have been able to obtain on the differences between the carbohydrate metabolism of healthy and crinkle potatoes show that in the early stages of the disease there is no significant difference between carbohydrate formation in the two cases, although the rate of transport appears to be delayed in the diseased material. This is certainly a curious result, as the crinkle plants present a definite clinical picture with clearing of the veins and mottling of the leaves. It would have been thought in the circumstances that significant differences would have been discovered, but beyond the fact that carbohydrate formation is accelerated in the diseased plants and also that the carbohydrate percentages are higher (even including the starch), the curves for hexose, sucrose and starch follow the same general trend.

Unlike the case of leaf-roll, the sugar of transport was found to be sucrose, and sucrose was always found to be abundant in the diseased petioles.

Differences, however, arise at the close of the growing season. Sucrose is abundant in the laminae of the crinkle plants, while hexose is abundant in the healthy. Starch has fallen to a very low value in both diseased and normal. With regard to the lowness of the starch values, Clements(2), working in America, found a similar result for the potato at the close of the season (see Fig. 18, p. 267). These results can be interpreted from our regressions. With the onset of senescence photosynthesis has practically ceased and the starch that is present in the leaf-blade is being rapidly hydrolysed to soluble sugars. In the healthy leaf the reaction is in the direction starch \rightarrow hexose \rightarrow sucrose, as well as starch \rightarrow sucrose, but principally the sucrose is formed from hexose. Sucrose in its turn is transported out of the leaf to the tubers. In the crinkle lamina, on the other hand, sucrose formation is taking place directly from starch and only to a small extent from hexose. We have in addition the fact that there is marked accumulation of sucrose in the diseased lamina, a result probably due to impediment of free transport in the petiole. It will be recalled that the sucrose curve for the crinkle petioles in Arran Victory, Series II, was suggestive of alternate damming up and then release of this sugar, and that the sucrose accumulated to a marked extent towards

the close of the day, while in Arran Victory, Series I, sucrose also showed a marked tendency to accumulate in the petioles. If this interpretation be correct, the accumulation of sucrose in the lamina becomes explicable and the fact that it is not converted back to starch as in leaf-roll is possibly due to the concentration not reaching the required level for the back reaction to take place, owing to its being periodically released down the petiole.

It is now possible to give an explanation of the fall in sucrose that has been found by practically every investigator of the carbohydrates of the leaf to take place later in the season. Since sucrose is the sugar of transport and is formed from hexose as well as in certain circumstances from starch, its presence and accumulation will depend on (1) the rate of formation, and (2) the rate of transport. If the rate of synthesis be great, *i.e.* when photosynthesis is active, the channel of transport may not be sufficient to cope with the supply. In these circumstances sucrose will tend to accumulate in the lamina of the leaf, and such is found to be the case in the early stages of development of the plant. Presumably in the early stages of growth photosynthesis will be more active than later on, and our own results in the present instance bear out this presumption, and sucrose will be abundant. Later, the rate of photosynthesis will fall, but sucrose still remains the sugar of transport and it will therefore follow that the synthesis of sucrose will not be as great as in the earlier stages of growth and development, and, moreover, its presence in the leaf-blade will be low because it is being continually withdrawn to the storage regions. In other words, the rate of withdrawal is greater than the rate of synthesis.

It is a well-known fact that the incidence of crinkle in a potato crop markedly reduces the yield. From our comparison of the carbohydrate metabolism in healthy and diseased material, it is clear that the results will not entirely explain this heavy loss. It is true that we have shown that there is interference with the channel of transport and that sucrose cannot travel in the diseased petiole with the same ease as in the healthy, but even in these circumstances, it is very doubtful if this will explain the magnitude of the losses sustained from crinkle. It is probable that other causes are more important in this connection, and it may be that differences in nitrogen metabolism and ash will be found to account for the poorness in yield of crinkle plants.

With regard to the presence of a latent virus in a variety, we have been unable to detect any significant difference in carbohydrate metabolism between healthy and apparently healthy, and the presence of a

virus disease in the latent state in a variety does not interfere with rate of photosynthesis.

V. SUMMARY.

The present investigation was concerned with the formation of carbohydrates in healthy and crinkle infected potato plants, and the nature of the sugar or sugars of transport. A series of observations were also made to determine whether there were any differences in the carbohydrate metabolism when a latent virus (paracrinkle) was present in a variety.

The varieties used were Arran Victory for crinkle and President for paracrinkle.

The disease was transmitted by stem-grafting to our virus-free units with Irish Chieftain for crinkle and King Edward for paracrinkle. All plants were grown in insect-proof greenhouses.

A series of experimental runs were then carried out at different times in the season. Samples of laminae and petioles were taken at hourly intervals over nineteen hours for crinkle and at two-hourly intervals over twenty-four hours for paracrinkle.

It was found that in the early stages of the disease there was no statistically significant difference between carbohydrate formation in healthy and crinkle plants. The chief differences that were detected were (1) that the percentages of all three carbohydrates that were estimated (hexose, sucrose and starch) were higher in the diseased material than in the healthy, and (2) that there was a time lag of approximately two hours between the maximum and minimum points in the crinkle and healthy laminae; the former preceding the latter.

Sucrose was found to be the sugar of transport in both cases.

In the later part of the season, significant differences were discovered in the two cases. Sucrose was found to show a marked accumulation in the diseased laminae, and it was ascertained that this sucrose was formed by the hydrolysis of starch, whereas in the healthy laminae the formation of sucrose was brought about by synthesis from hexose, which in turn was formed from hydrolysis of starch. A certain amount of the sucrose was also discovered to be produced directly from starch.

It was also ascertained that sucrose was still the sugar of transport, but evidence was obtained to show that its passage down the diseased petioles was not so readily effected as in the healthy.

The low yields from crinkle infected potatoes is discussed in the light of these results.

The presence of a latent virus in a variety was shown to produce no significant difference in carbohydrate formation, either at the beginning or the close of the growing season.

This investigation was carried out at the Station of the Scottish Society for Research in Plant Breeding, Corstorphine, Edinburgh, under a scheme financed by the Empire Marketing Board. We would like to take this opportunity of thanking the Director, Mr William Robb, F.R.S.E., and Dr A. Nelson, F.R.S.E., for their helpful criticism in a number of different directions.

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STUDIES IN THE PHYSIOLOGY OF THE VIRUS DISEASES OF THE POTATO

III. A COMPARISON OF THE NITROGEN METABOLISM OF NORMAL WITH THAT OF LEAF-ROLL POTATOES

By EUSTACE BARTON-WRIGHT, M.Sc.
AND ALAN M^cBAIN, B.Sc.

*(From the Scottish Society for Research in Plant Breeding,
Corstorphine, Edinburgh.)*

(With 17 Text-figures.)

I. INTRODUCTION.

IN a continuation of our survey of the physiology of the virus diseases of the potato, we have made a series of observations on the nitrogen relations existing in healthy and leaf-roll infected material, which are embodied in the present communication.

The investigation of the nitrogen metabolism of the green plant is beset with difficulties, not the least of which is the fact that only groups of substances can be estimated and not single entities. Moreover, many of the nitrogen compounds present in the plant are of unknown nature and constitution, and further, even the seat of protein synthesis is still in dispute.

In the circumstances, it became necessary to settle a number of subsidiary problems in the healthy material, before an adequate comparison could be made with diseased stocks. Furthermore, we were considerably hampered by the fact that the existing literature on nitrogen metabolism of the green plant is contradictory and controversial.

Our colleague, Mr George Cockerham, B.Sc., of the sub-station, Huntly, Aberdeenshire, has already published a preliminary communication⁽⁸⁾ on the diurnal variations in total nitrogen in the laminae and petioles of healthy and leaf-roll potatoes, and the present communication is a continuation of this work. The relevant literature on the nitrogen content of leaf-roll potatoes is summarised in Cockerham's paper, and we only propose to summarise the results of one paper here. It has been claimed by Schweizer^(21, 22) that in leaf-roll tubers there is a migration of all protein to the shoots, and that this complete migration of protein

leads to a cessation of diastatic activity in the diseased tubers, with the result that hydrolysis of starch and consequent translocation of soluble sugars to the newly developing shoots is seriously affected. He also claimed that shortage of protein is responsible for phloem necrosis, which is a characteristic of this disease, and stated that there is a marked accumulation of nitrate in the laminae of the diseased plants, until ultimately the nitrate content of these organs exceeds that of the stems. He concluded as a result of this work that leaf-roll is a disease of deranged protein metabolism. We have been unable to substantiate these latter statements.

II. METHODS.

(1) *Material.*

The same potato varieties as those employed by Cockerham were used in this investigation, namely, Arran Victory and President. As we have stated elsewhere⁽³⁾, Arran Victory, although susceptible to leaf-roll, is not affected to such a marked extent as President, which is particularly intolerant of the disease.

(2) *Transmission.*

The diseased material was obtained from our stocks of leaf-roll tubers which had been infected in the previous season by means of *Myzus persicae*.

(3) *Duration of experiments.*

The various nitrogen fractions as well as total nitrogen were estimated over twenty-four hour periods at different times in the growing season, the samples of leaves and petioles for these diurnal experiments being gathered at two-hourly intervals. All diurnal determinations commenced at 11 p.m. and were concluded at 9 p.m., G.M.T. Seasonal observations were also made on the two varieties by taking samples at weekly intervals over the growing period.

(4) *Grading.*

Grading was carried out as in our other work⁽³⁾ and sampling in the same way. Fifty healthy and fifty leaf-roll plants were used for each diurnal series. It should be mentioned here that all plants for this work were grown in insect-proof greenhouses.

(5) *Preparation of material.*

After each collection, laminae were separated from the petioles and each was then treated as a separate unit. The mid-rib and principal veins

were rejected. The material was killed and worked up by the methods described by Thomas⁽²³⁾, which proved to be very satisfactory.

(6) *Methods of analysis.*

(a) *Carbohydrates.* These were determined by the Schaffer-Hartman micro-method.

(b) *Total nitrogen* was estimated on the dried material by Ranker's modification of the Kjeldahl method⁽²⁰⁾. This includes nitrate nitrogen.

(c) *Total crystalloid nitrogen* (non-protein N) was determined on the solutions obtained after extraction of the dried material and clearing with colloidal ferric hydroxide (by the method described by Thomas). 25 c.c. and 50 c.c. lots of cleared extract were made acid to litmus with acetic acid and evaporated to dryness. The nitrogen present was then estimated as for total nitrogen.

(d) *Ammonia nitrogen* was estimated on 25 c.c. of cleared extract by making the solution alkaline with sodium carbonate and aerating into N/100 H₂SO₄ by the Folin method.

(e) *Amino-acid nitrogen* was found by the formol method (cf. Cole⁽⁹⁾).

(f) *Amide nitrogen* was determined on 25 c.c. of cleared extract, to which was added 4 c.c. of 50 per cent. sulphuric acid and the whole then made up to 50 c.c. with water. The mixture was gently refluxed for four hours, made alkaline and estimated by the Folin aeration method.

(g) *Nitrate nitrogen* was estimated on the duplicate samples from the ammonia determinations, using Devarda's alloy and aerating for twenty hours.

Since in the present investigation we were concerned with the diurnal fluctuations of definite chemical substances and not merely with amide N and amino N, the results are expressed in the following terms:

(i) *Asparagine N.* The assumption was made that the whole of the amide N is present in the form of amides of the type of asparagine. Asparagine N is therefore taken as being twice amide N.

(ii) *Amino-acid N.* This was found by deducting ammonia N, and amino N due to amide, from the total amino N recorded by the formol method.

(iii) *Residual N.* We have used this term to cover the fraction of crystalloid N not accounted for by the sum of the asparagine N, amino-acid N, nitrate N and ammonia N. We have made no attempt to discover the nature of the substances included.

(iv) *Protein N.* This was obtained by deducting crystalloid N (non-protein N) from total nitrogen.

(7) *Expression of results.*

We have calculated our results for the diurnal determinations as a percentage of the residual dry weight, except where otherwise stated. The seasonal determinations have been calculated as a percentage of the total nitrogen.

(8) *Statistical treatment.*

Wherever necessary the data have been submitted to statistical examination, and the various direct and partial correlation coefficients, as well as regressions, calculated.

III. EXPERIMENTAL OBSERVATIONS.

The diurnal observations on Arran Victory were carried out on May 16th, 1932, and those on President on July 27th, 1932. The results, with minor differences, confirm one another, and on this account it will be more convenient to describe the results obtained for the two sets of healthy material together, and to follow these with the results obtained for leaf-roll material.

DIURNAL VARIATIONS IN HEALTHY ARRAN VICTORY,
SERIES I A (MAY 16TH, 1932).(1) *Diurnal variations in total nitrogen of laminae and petioles.*

The variations in total nitrogen in lamina and petiole were followed at two-hourly intervals. The results expressed as the weight of total nitrogen per 100 gm. residual dry weight are shown graphically in Fig. 1. The individual results are expressed as a percentage of the mean of the total nitrogen, and, to show with greater clarity the general drift in time in the two tissues, we have plotted in the figures the means of successive pairs of observations.

The figures completely confirm the findings of Cockerham with material collected in 1931. In the lamina there is a well-marked diurnal variation in total nitrogen. The curve rises to a first maximum at 6 a.m. and then falls to a minimum at 10 a.m., and rises to a second maximum at 2 p.m. It is clear from the curve that the day values are greater than the night values. In the petiole, the curve also rises sharply to a maximum at 6 a.m., falls to a minimum at 10 a.m., and rises to a second maximum at 2 p.m., and fluctuations in the lamina are faithfully followed in the petiole.

In the lamina we interpret these results as showing synthesis in the early part of the day, followed by export of organic nitrogen. The nature

of our material precluded the possibility of tissue differentiation, so that in the petiole we are dealing with the mean results of three different tissues, xylem, phloem and ground parenchyma, and it becomes a matter

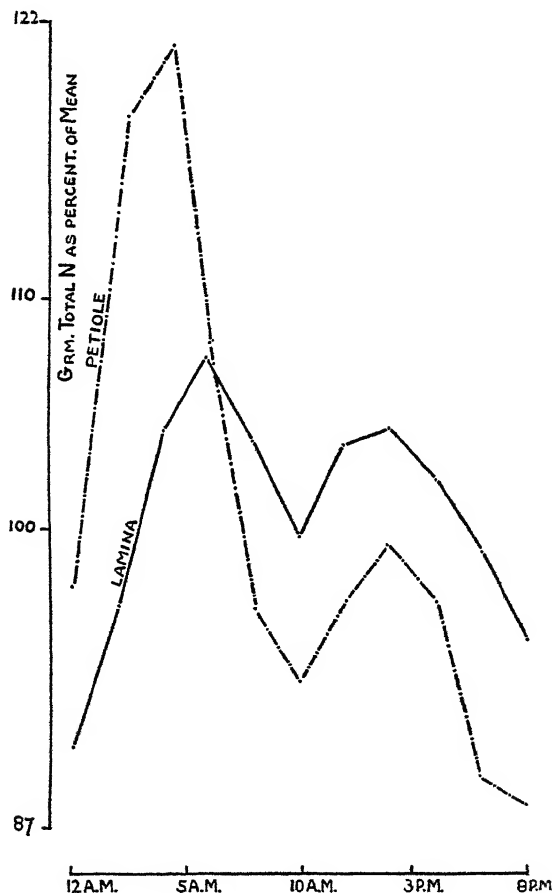


Fig. 1. Diurnal fluctuations in the total nitrogen content of laminae and petioles of healthy Arran Victory. Results expressed per 100 gm. of residual dry weight. Plotted as a percentage of the mean value for each tissue.

of difficulty to interpret the results. The early rise in the petiolar curve we attribute to the entrance of nitrate nitrogen. Similarly, the fall in the petiolar curve after 6 a.m. we attribute to fall in nitrate nitrogen, but the second maximum in the petiolar curve we consider to be due to

export of organic nitrogen out of the lamina. The reasons for this interpretation are given below.

It was found by Chibnall(6) that there was a diurnal variation in nitrogen in the leaves of the runner bean when the results were expressed

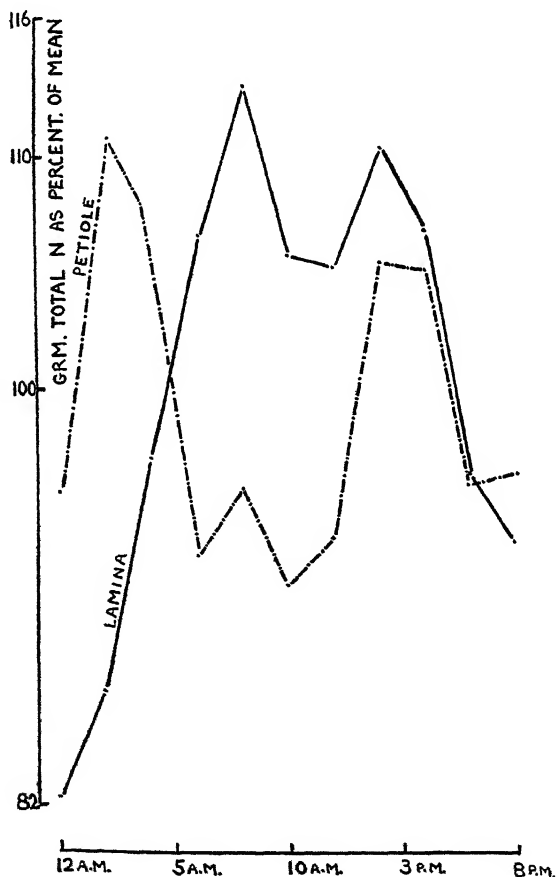


Fig. 2. Diurnal fluctuations in the total nitrogen content of laminae and petioles of healthy Arran Victory. Results expressed per 100 gm. of fresh weight. Plotted as a percentage of the mean value for each tissue.

on a fresh weight basis. Maskell and Mason(12) have also found a diurnal variation in total nitrogen in the leaf of the cotton plant as well as in the bark when the results were calculated as a percentage of the fresh weight, but when the basis of residual dry weight was used, the diurnal variation in the bark was not statistically significant and variation in time was not

much greater than variation due to sampling. We have also in this instance calculated our results as a percentage of the fresh weight and these are graphically expressed in Fig. 2. It will be seen that for the lamina there is again a well-marked diurnal variation in total nitrogen, the values rising during the day and decreasing during the night, although the first maximum is not reached until 8 a.m., two hours later than on the residual dry weight basis, and the fall is not so marked as in Fig. 1. The general trend of the curve, however, is similar to that obtained on a residual dry weight basis. In the petiolar curve, the first maximum is reached six hours earlier than in Fig. 1, and thereafter does not follow the lamina curve with the same faithfulness as on a residual dry weight basis, although there is a marked rise in the petiolar curve at 10 a.m., as was shown in Fig. 1.

Thus, on either a residual dry weight or a fresh weight basis there is a well-marked diurnal variation of nitrogen shown by both lamina and petiole, indicating that in the lamina there is synthesis by day and export by night.

(2) *Diurnal variations in ammonia N, asparagine N, amino-acid N and nitrate N in the lamina (Arran Victory, Series Ia).*

In Fig. 3 the diurnal fluctuations in ammonia N, asparagine N, amino-acid N and nitrate N are graphically shown as a percentage of the residual dry weight.

Ammonia N after an initial rise falls over the greater part of the experimental period, rising again towards the close of the day. Asparagine N, on the whole, is rising throughout the day, and finally falls markedly at the close of the experimental period, while amino-acid N rises after the first four hours, remains at a maximum for six hours and then decreases. The nitrate N curve falls sharply for the first six hours, and after showing two maxima at 7 a.m. and 1 p.m., respectively, falls away to the close of the day. It would therefore appear that nitrate N accumulates during the night and decreases during the day.

Leaving out of consideration for the moment the behaviour of nitrate N (we will return to this question in the discussion on the formation of protein N), we interpret these results as showing a conversion of ammonia N to asparagine N and amino-acid N. Support for this view is given by the various direct and partial correlation coefficients as well as regressions. Of the various direct correlations given in Table I, the only one that is statistically significant is that between ammonia N and asparagine N. If the reactions between these various fractions is proceeding independently, the partial correlations should all show a rise over the values given

above. Calling ammonia N, *A*, asparagine N, *B*, amino-acid N, *C* and nitrate N, *D*, the partial correlations are given in Table II.

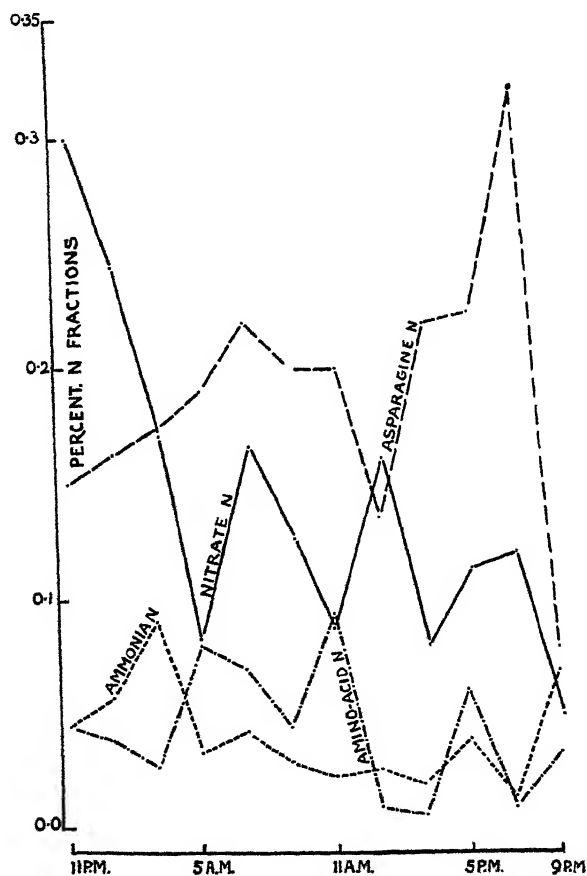


Fig. 3. Diurnal fluctuations in ammonia N, asparagine N, amino-acid N and nitrate N in the laminae of healthy Arran Victory. Results expressed as a percentage of residual dry weight.

Table I.

Ammonia/asparagine N	$r = -0.5866^*$
Ammonia/amino-acid N	$r = +0.0665$
Ammonia/nitrate N	$r = +0.2103$
Asparagine/amino-acid N	$r = +0.3851$
Asparagine/nitrate N	$r = +0.0561$
Amino-acid/nitrate N	$r = -0.1820$

* We have again used *P* instead of calculating the probable error (see (2)).

The significant correlations are shown in heavy type.

Table II.

$c^r_{AB} = -0.6645$	$B^r_{AC} = +0.3898$	$B^r_{AD} = +0.3009$
$D^r_{AB} = -0.6165$	$D^r_{AC} = +0.1087$	$C^r_{AD} = +0.2183$
$CD^r_{AB} = -0.7254$	$BD^r_{AC} = +0.5054$	$BC^r_{AD} = +0.4307$
$A^r_{BC} = +0.5248$	$A^r_{BD} = +0.2272$	$A^r_{CD} = -0.2009$
$D^r_{BC} = +0.4028$	$C^r_{BD} = +0.1341$	$B^r_{CD} = -0.2205$
$AD^r_{BC} = +0.6001$	$AC^r_{BD} = +0.3987$	$AB^r_{CD} = -0.3860$

It will be seen that the original direct correlation between ammonia N and asparagine N rises with elimination of amino-acid N and nitrate N, and further, that the correlation between asparagine N and amino-acid N also reaches statistical significance when ammonia N and nitrate N are eliminated. In the same way the correlation between ammonia N and amino-acid N becomes almost significant when asparagine N and nitrate N are eliminated. It would thus appear that there is a close relationship between ammonia N and asparagine N, asparagine N and amino-acid N, and amino-acid N and ammonia N. The corresponding regressions are given below:

$$A = 0.0516 - 0.1967B + 0.3309C + 0.1032D \quad \dots(1),$$

$$B = 0.1941 - 2.675A + 1.445C + 0.3523D \quad \dots(2),$$

$$C = -0.0150 + 0.7718A + 0.2480B + 0.1413D \quad \dots(3).$$

In none of the three equations given above does nitrate N play any significant part in the formation of ammonia N, asparagine N, or amino-acid N. From (2) formation of asparagine N is due to decrease of ammonia N, while apparently there is a reversible reaction between amino-acid N and asparagine N. Lastly, from (3) increase or decrease in amino-acid N is dependent on increase or decrease of ammonia N, while from (1) increase or decrease of ammonia N is dependent on amino-acid N to a small extent.

(3) *Diurnal variations in protein N and residual N in the lamina and nitrate N in the petiole (Arran Victory, Series Ia).*

We have plotted in Fig. 4 the diurnal variations in protein N and residual N in the lamina and nitrate N in the petiole. It will be seen from the curves that nitrate N in the petiole is increasing rapidly in the early hours of the morning, and it is on this account that we have interpreted the rise in the early hours of the morning in the curve for total nitrogen in the petiole as being due to nitrate N. The nitrate N curve falls away towards the middle part of the experimental period and then commences to rise once more towards the close of the day. The curve for protein N also rises in the early part of the day and reaches its first maximum at

7 a.m., falls, and rises to a second maximum at 1 p.m. Residual N, on the other hand, rapidly reaches a maximum at 3 a.m., and also reaches a maximum at the close of the experimental period. For the sake of comparison, we have included in the figures the curve for nitrate N in the

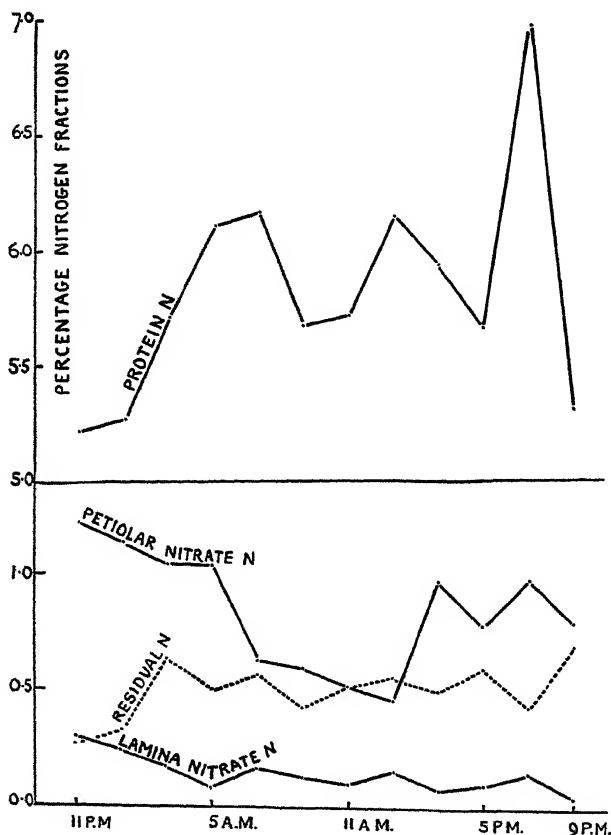


Fig. 4. Diurnal fluctuations in protein N, residual N and nitrate N in the laminae of healthy Arran Victory and nitrate N in the petioles. Results expressed as a percentage of residual dry weight.

lamina. The low percentage of lamina nitrate N in comparison with petiolar nitrate N should be observed. It is evident that entering nitrate N is rapidly used up in the lamina. In Table III we give the direct correlations between protein N (*P*) and residual N (*R*) and the various nitrogen fractions described above.

Table III.

Protein/ammonia N	$r = -0.4938$
Protein/asparagine N	$r = -0.4203$
Protein/amino-acid N	$r = +0.0179$
Protein/nitrate N	$r = -0.5973$
Residual/ammonia N	$r = +0.3175$
Residual/asparagine N	$r = -0.1727$
Residual/amino-acid N	$r = +0.1523$
Residual/nitrate N	$r = -0.7145$
Residual/protein N	$r = +0.3661$

Of these direct correlations, two are statistically significant, protein/nitrate N and residual/nitrate N, and both are negative in sign. It should also be noted that the protein/ammonia N correlation, although not statistically significant, is high and negative.

It will be recalled that nitrate N in the lamina (Fig. 3) is on the whole falling during the day, whereas protein N is increasing during the day. Residual N also reaches a maximum in the early part of the experimental period and also at the close of the day. From the nature of the correlations and their sign, it would seem that both protein N and residual N are directly formed from nitrate N, and that the other nitrogen fractions play no significant part in their synthesis. There is also a close relationship between ammonia N and protein N. The direct correlation is high and the partial correlations reach statistical significance. Thus, for example, when residual N is eliminated, $Rr_{AP} = -0.6942$, or with amino-acid N and residual N eliminated, $ORr_{AP} = -0.6940$, and the partial correlations are fully significant. We have not considered it necessary to give all the partial correlations between protein N and ammonia N, as it would only tend to confuse the issue. The partial correlations between residual N and ammonia N also in a number of cases became significant, but positive in sign. Thus, with protein N eliminated, $Pr_{AR} = +0.6156$, and with nitrate N eliminated, $Dr_{AR} = +0.6837$. It is evident that the relationship between ammonia N and protein N is close, and we suggest in the direction protein N \rightarrow ammonia N.

The partial regressions between protein N, residual N and nitrate N are given below:

$$R = 0.9114 - 1.371D - 0.0374P \quad \dots(1),$$

$$P = 6.488 - 3.313D - 0.3369R \quad \dots(2).$$

It is clear from these equations that any interconversion of residual N and protein N is not significant, but that both residual N and protein N are formed from nitrate N.

DIURNAL VARIATIONS IN HEALTHY PRESIDENT, SERIES II A
(JULY 27TH, 1932).

(1) *Diurnal variations in total nitrogen of laminae and petioles.*

Variations in total nitrogen in laminae and petioles of healthy President are shown in Fig. 5. The diurnal variation in total nitrogen in the lamina

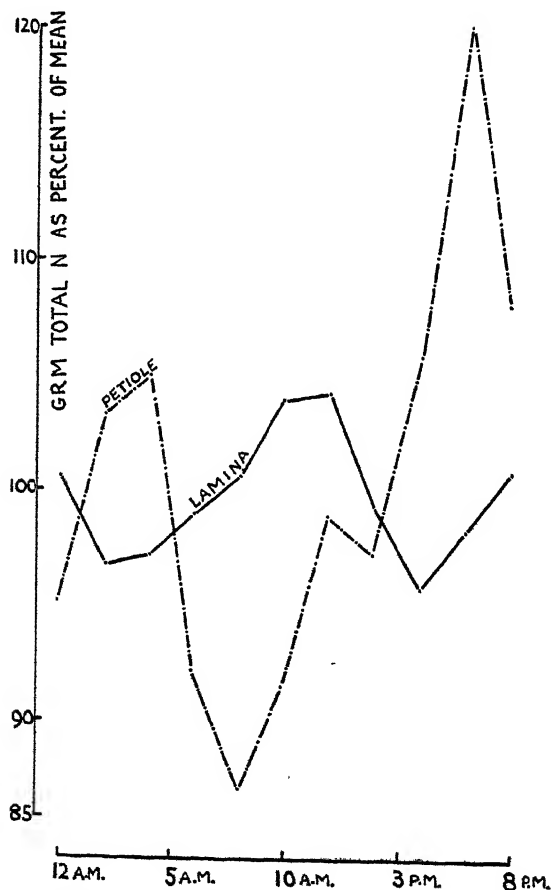


Fig. 5. Diurnal fluctuations in the total nitrogen content of laminae and petioles of healthy President. Results expressed per 100 gm. of residual dry weight. Plotted as a percentage of the mean value for each tissue.

is particularly clear, and again confirms Cockerham's results. As in the Arran Victory series (Series Ia), there is a preliminary rise in petiolar

total nitrogen in the early hours of the morning and this is followed by a fall. The subsequent rise in petiolar nitrogen lags behind that of the lamina and, in this instance, it is not until six hours after lamina total nitrogen has commenced to rise that petiolar total nitrogen also commences to mount and continues till a maximum is reached ten hours later.

We again interpret these results as showing synthesis of nitrogenous compounds in the lamina during the day and export during the night (see above). For the petiole the preliminary rise in total nitrogen is interpreted as being due to import of nitrate N and the second rise as being due to export of elaborated organic nitrogen.

(2) *Diurnal variations in ammonia N, asparagine N, amino-acid N and nitrate N in the lamina (President, Series IIa).*

We have plotted in Fig. 6 the diurnal variations in ammonia N, asparagine N, amino-acid N and nitrate N.

Ammonia N after remaining relatively constant for four hours rises sharply to a maximum at 5 a.m. and then falls away to the close of the experimental period. Asparagine N falls sharply at first, rapidly reaches a maximum and then falls away continuously to the end of the day. Amino-acid N rises in the early part of the day, fluctuates and then finally drops towards the close of the experimental period. The curve for nitrate N shows accumulation of nitrate at night in a marked manner, a fall during the greater part of the day and then accumulation once more.

In Table IV we give the direct correlation coefficients between these various fractions:

Table IV.

Ammonia/asparagine N	$r = -0.1200$
Ammonia/amino-acid N	$r = +0.7050$
Ammonia/nitrate N	$r = -0.0268$
Asparagine/amino-acid N	$r = -0.3195$
Asparagine/nitrate N	$r = +0.1649$
Amino-acid/nitrate N	$r = -0.0761$

The only significant correlation here is that between amino-acid N and ammonia N. The correlation between ammonia N and asparagine N, although negative in sign, is not statistically significant. On the other hand, the asparagine N curve follows a very similar course to that of ammonia N, with the added fact that it seems to lag behind it. We have already shown (Arran Victory, Series Ia) that the relation between ammonia N and asparagine N is very close. If there be any relation in this case between these two fractions, then the correlation should be increased if we advance the values a period. When the correlation coefficient

between ammonia N and asparagine N is calculated with the values shifted two hours, it becomes statistically significant ($r = +0.8896$). Although positive in sign, from the fact that asparagine N lags behind ammonia N, we suggest that ammonia N is converted to asparagine N (see in this connection Arran Victory, Series Ia). The correlation between ammonia N

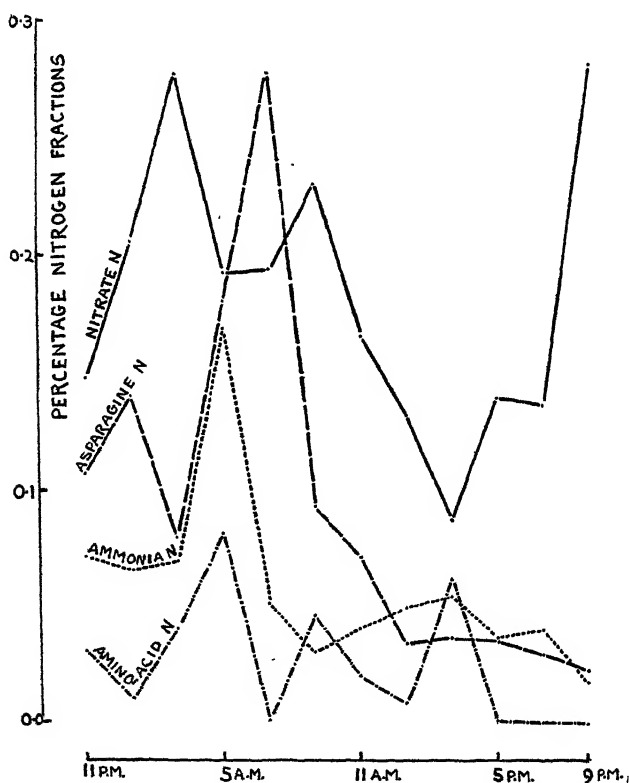


Fig. 6. Diurnal fluctuations in ammonia N, asparagine N, amino-acid N and nitrate N in the laminae of healthy President. Results expressed as a percentage of residual dry weight.

and amino-acid N is statistically significant. In Arran Victory, Series Ia, the direct correlation in this case was not significant, but increased almost to significance when asparagine N and nitrate N were eliminated ($_{BD}r_{AC} = +0.5054$, see Table II). In this series of observations the partial correlation with asparagine N and nitrate N eliminated increases slightly over the direct correlation ($_{BD}r_{AC} = +0.7084$), *i.e.* the reaction

between ammonia N and amino-acid N is proceeding independently of the presence of other factors. Using the same notation as in Arran Victory, Series Ia, the partial regressions for ammonia N, asparagine N and amino-acid N are given below:

$$A = 0.0303 + 0.0598B + 1.074C - 0.0286D \quad \dots(1),$$

$$B = 0.0547 + 0.3874A - 1.255C + 0.1816D \quad \dots(2),$$

$$C = 0.0069 + 0.4673A - 0.0844B - 0.0085D \quad \dots(3).$$

As in the case of the Arran Victory figures, nitrate N appears to play no significant part in the formation of either ammonia N, asparagine N or amino-acid N. Asparagine N formation appears to depend on decrease of amino-acid N (equation 2), but, as we have already seen, the relationship between ammonia N and asparagine N is close and asparagine N formation lags behind that of ammonia (Fig. 6). Thus, asparagine formation in this case falls into line with Arran Victory. Lastly, increase or decrease of amino-acid N is dependent on increase or decrease of ammonia N.

(3) *Diurnal variations in protein N and residual N in the lamina and nitrate N in the petiole (President, Series IIa).*

Fig. 7 shows the diurnal variations in protein N and residual N in the lamina and nitrate N in the petiole. As in Arran Victory, nitrate N in the petiole increases rapidly in the early part of the experimental period and then falls away, rises and falls once more. Protein N, after an initial fall, rises to a maximum at 5 a.m., falls, and then fluctuates. We have again included in the figures the curve for nitrate N in the lamina. Here, as in Arran Victory, the lamina nitrate percentages are very low in comparison with entering petiolar nitrate N.

In Table V we give the various direct correlation coefficients between residual N, protein N and other nitrogen fractions:

Table V.

Protein/ammonia N	$r = -0.5560$
Protein/asparagine N	$r = -0.1554$
Protein/amino-acid N	$r = -0.6232$
Protein/nitrate N	$r = -0.0160$
Residual/ammonia N	$r = +0.5217$
Residual/asparagine N	$r = -0.4633$
Residual/amino-acid N	$r = +0.6434$
Residual/nitrate N	$r = -0.0819$
Residual/protein N	$r = -0.3521$

Of these direct correlation coefficients, protein/ammonia N, protein/amino-acid N, residual/ammonia N and residual/amino-acid N are statistically

significant. It will be remembered that in the Arran Victory figures, the direct correlation between protein/ammonia N was high but not significant, but the partial correlation with various factors eliminated became so. It should also be noted that the residual/ammonia N correlation is statistically significant, whereas in the case of Arran Victory it became

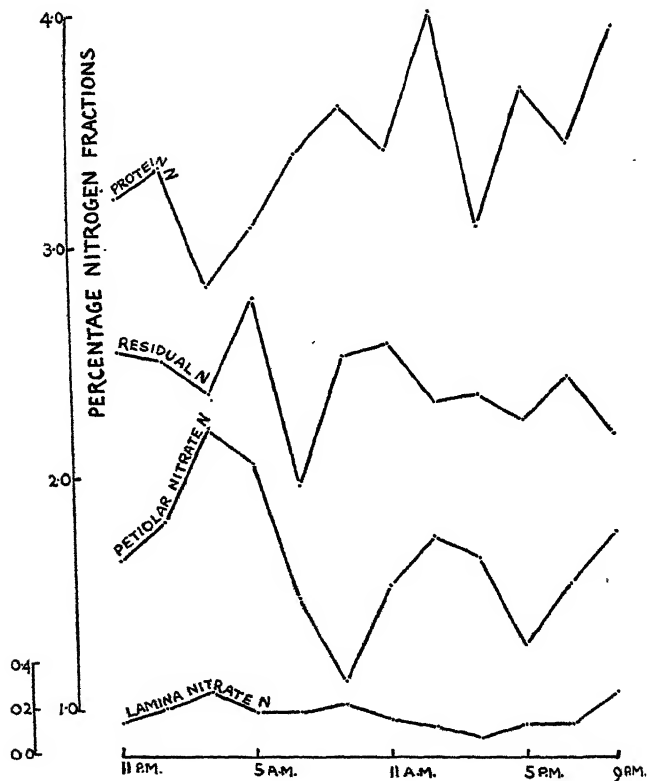


Fig. 7. Diurnal fluctuations in protein N, residual N and nitrate N in the healthy laminae of President, together with nitrate N in the petioles. Results expressed as a percentage of residual dry weight.

so when the partial correlations were calculated. On the other hand, the correlations amino-acid N and protein N, as well as residual N, are statistically significant. This is not in agreement with our Arran Victory figures. But actually the relationship here between protein N, residual N and amino-acid N is through ammonia, for when the partial correlation coefficients are calculated with ammonia N eliminated, the direct

correlations fall from statistical significance to statistical non-significance. Thus, ${}_A r_{CP} = -0.3992$ and ${}_A r_{CR} = 0.4554$. It is evident from these partial correlations that any relationship that may exist between these three fractions is through ammonia, and the results fall into line with Arran Victory, Series Ia.

The correlation between protein N and nitrate N and residual N and nitrate N in this case is very low. But comparison of Fig. 7 with Fig. 6 shows that protein N, on the whole, is increasing between 3 a.m. and 1 p.m., while nitrate N is, on the whole, decreasing during this period. Similarly, in the case of residual N, while nitrate N is at a minimum, residual N is at a maximum. It will be recalled that the direct correlation between ammonia N and asparagine N in this series was not statistically significant, but became so when a shift of two hours was made, *i.e.* formation of asparagine N lagged behind that of ammonia N. When a shift of two hours is made in protein N, residual N and nitrate N, the direct correlations increase, and that between residual N and nitrate N almost reaches significance:

$$\text{Protein/nitrate N} \quad r = -0.4042,$$

$$\text{Residual/nitrate N} \quad r = 0.5062.$$

That protein formation takes place directly from nitrate N in President, as we showed for Arran Victory, is also confirmed by our leaf-roll results (see below, Section VI, (3), p. 575).

Again, as in Arran Victory, so in President, the regressions show that both residual N and protein N are significantly related to nitrate N, but that there is apparently no interconversion of protein N and residual N:

$$R = 2.471 + 1.545D - 0.1010P \quad \dots\dots(1),$$

$$P = 4.588 - 1.885D - 0.3461R \quad \dots\dots(2).$$

IV. SEASONAL VARIATIONS.

An investigation was made of the fluctuations of the various nitrogen fractions in the lamina of Arran Victory and President over the total growing season. Samples were collected at weekly intervals at the same time (9 a.m. G.M.T.). The results are expressed as a percentage of the total nitrogen.

In Fig. 8 we show the results graphically expressed for Arran Victory. The first point to notice is that ammonia N, asparagine N and amino-acid N are all very small in amount. This result is in marked contrast to that found by McKie⁽¹¹⁾ for lupin seedlings over the growing period. In the

lupin seedling it was discovered that over a growing season of 81 days, protein, insoluble nitrogen compounds and asparagine represent by far the largest components of the total nitrogen. The most conspicuous feature of McKie's curves was the behaviour of asparagine, which increased rapidly to a maximum and then fell away, and with this fall in asparagine there was a corresponding increase in protein. McKie follows

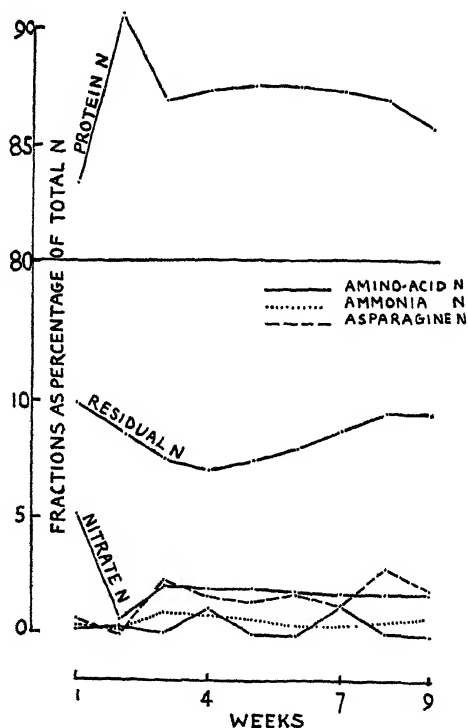


Fig. 8. Seasonal fluctuations in protein N, residual N, nitrate N, ammonia N, asparagine N and amino-acid N in the laminae of healthy Arran Victory. Samples gathered at weekly intervals and results expressed as a percentage of total nitrogen.

Chibnall (5) in considering that asparagine is the form in which nitrogen is translocated in the plant and is the product whereby nitrogen is conveyed from regions of protein catabolism to those of re-synthesis.

As far as our own results are concerned, we are not in agreement with this view. It is possible that asparagine plays an all-important part in protein synthesis and nitrogen translocation in the Leguminosae, but this is not the case for the potato. In Arran Victory (Fig. 8) there is a

heavy fall in nitrate N over the first week of growth and a corresponding rise in protein N. In the second week the nitrate N rises and thereafter remains constant over the rest of the season, the protein N falls and then remains constant. Residual N falls slowly for the first four weeks and then rises equally slowly to the end of the growth period. We interpret these results as showing that protein synthesis is most active in Arran Victory in the first few days of growth, and the behaviour of nitrate N in this connection bears out our suggestion that protein N is directly formed from nitrate N. With regard to residual N, it has been suggested by Maskell and Mason (13, 14, 15, 16) that this fraction forms the head for transport of nitrogen in the cotton plant. This would appear to be correct, since in the healthy plant there is but little fluctuation over the whole season, whereas in the leaf-roll plant (see pp. 583-4, Figs. 16, 17) there is a marked accumulation of residual N at the close of the growing period.

Turning now to the case of President (Fig. 9), there is again a heavy fall in nitrate N at the beginning of the season, after an initial increase at the end of the first week. Nitrate N thereafter falls away to the end of the growth period. Protein N, on the other hand, after an initial fall increases slowly throughout the season, while residual N, like nitrate N, falls away gradually. The fact that residual N falls away in such a marked manner throughout the season again suggests that it is being translocated out of the lamina. We have not included in this figure the values for ammonia N, asparagine N and amino-acid N, since these, as in Arran Victory, were low in amount, especially asparagine N, which in President is very much lower than in Arran Victory. The behaviour of nitrate N, residual N and protein N in President, however, is similar in its general trend with that of Arran Victory, and we interpret the results in the same way.

V. DISCUSSION.

From our observations on the diurnal variations of total nitrogen as well as on the nitrogen fractions in Arran Victory and President, it seems clear that the lamina of the potato plant is the chief seat of nitrogen synthesis. The primary source of nitrogen from which complex nitrogenous compounds are elaborated in the potato is nitrate nitrogen. Nitrate nitrogen enters the lamina via the petiole, and it is evident from the relative amounts present in the two organs that its conversion to elaborated nitrogen is swift. It will be recalled that we found accumulation of nitrate nitrogen during the night in the lamina and that the

amount fell during the day, showing that synthesis of protein is greater by day than by night.

The question arises as to the nature of the various steps concerned in the process, whereby simple nitrate is converted to complex organic nitrogen in the form of protein.

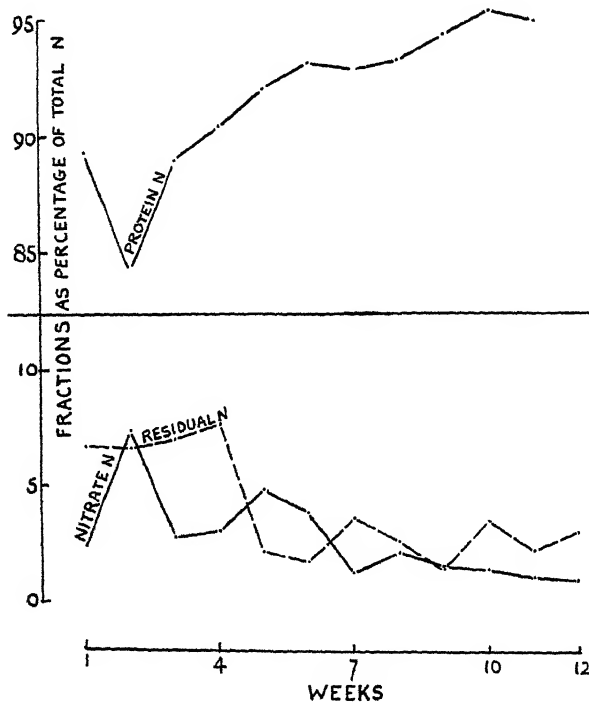
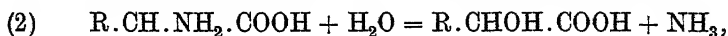
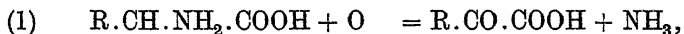


Fig. 9. Seasonal fluctuations in protein N, residual N and nitrate N in the laminae of healthy President. Samples gathered at weekly intervals and results expressed as a percentage of total nitrogen.

A great deal of stress has been laid in the past on the function of asparagine in the green plant. This is possibly due to the fact that a large number of investigations on the nitrogen relations of the green plant have been conducted on various members of the Leguminosae, and asparagine is undoubtedly present in large amount in this family. Chibnall (7), for example, considers that there is a continuous production of asparagine from protein in the mature green leaf, and that in plant metabolism asparagine plays the rôle of a translocatory substance and is the chief

medium whereby nitrogen, in a form suitable for subsequent re-synthesis, can be conveyed from one part of the plant to another. The evidence produced in this investigation does not agree with this suggestion, although we do not suggest that asparagine may not play the part assigned to it by Chibnall (5) as far as the Leguminosae are concerned. In the potato asparagine N does not bulk so large among the various nitrogen fractions, and in point of fact in President is very small in amount. Moreover, we were quite unable to find any relationship between protein N and asparagine N. On the other hand, a close connection was found between ammonia N and asparagine N, and from the diurnal fluctuations of these two fractions we showed that ammonia N was passing to asparagine N. Prianschnikow (17, 18, 19) has put forward the view that asparagine is formed as a temporary nitrogen reserve whenever there occurs, through some derangement of normal nitrogen metabolism, an excess of ammonia which is toxic to the plant. This toxic ammonia is rendered harmless by condensation with aspartic acid to give asparagine. We are largely in agreement with this view, and our diurnal curves certainly bear out this suggestion. Ammonia formed, either from oxidation of amino-acids or hydrolysis of proteins, is not allowed to accumulate but is converted to harmless asparagine. Moreover, Maskell and Mason (13, 14, 15) have found that in the cotton plant there are marked negative gradients of asparagine nitrogen in the stem, so it would appear that asparagine is not a translocatory product in this plant.

With regard to ammonia formation, we have discovered two sources of formation: (1) from amino-acids, and (2) from protein. Ammonia formation from amino-acids can take place in several ways, such as direct oxidation or hydration:



and in the potato tuber a mechanism is known whereby deamination of amino-acids is brought about by the catechol-oxidase system. Ammonia can also be formed from protein. For example, tripeptides of the type of glycyl-*L*-asparaginy-*L*-leucine yield on hydrolysis free ammonia, and since many proteins yield free ammonia on hydrolysis, this would seem to suggest that a certain number of acid-amide linkages are present in the protein molecule. We have also shown that increase or decrease of ammonia leads to increase or decrease of amino-acids, so that there is presumably amination of organic acids to amino-acids and deamination of amino-acids to ammonia and organic acids.

At the present time there are two possible hypotheses in the field to account for the synthesis of protein in the green plant. On the first hypothesis, separate synthesis of each constituent amino-acid of the protein molecule takes place, and, later, by a series of condensations, these amino-acids condense to form peptides, peptones, proteoses and proteins. On the second hypothesis, amino-acids are not synthesised separately in this way, but large constituent molecules of protein are condensed *en bloc* from compounds of greater simplicity than amino-acids.

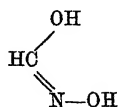
The chief difficulty that has always arisen in connection with the first hypothesis described above is the introduction of the amino-group. In the present investigation we were quite unable to find any direct relationship between amino-acids and proteins, but it will be recalled that a well-marked relationship was found between nitrate N and protein N.

Abderhalden has put forward the view that the protein molecule is built up of a number of di-ketopiperazine-containing complexes which are associated or held together by secondary valencies. Several such have been isolated from protein hydrolysates. On the assumption that protein formation takes place by condensation *en bloc*, the amino-acids must be regarded as secondary products formed either directly from protein, or else by a secondary process from ammonia produced by degradation of protein.

The evidence available from this investigation points to this second hypothesis as being the correct one for protein synthesis in the green plant. Nitrates, however, are relatively stable bodies and therefore must undergo some preliminary change before they are brought into a sufficiently reactive state to condense with other products to form protein and residual nitrogen. The usual suggestion has been that nitrate is first reduced to nitrite. The presence of nitrite in leaves has been reported by a number of investigators and Anderson(1) has described a nitrate-reducing mechanism in *Mercurialis perennis*. Similarly, Eckerson(10) has been able to demonstrate the presence of a nitrate-reducing mechanism in the tomato. She showed that the expressed sap from tomato leaves is able to reduce nitrates to nitrites and eventually to give rise to ammonia, and this process can take place either in light or darkness. Iron was found to be an important factor in the reduction process.

Baudisch(4) and Baly, Heilbron and Hudson(2) have put forward theories with regard to protein synthesis in the plant. According to Baudisch, the cholera bacillus has the power of accumulating iron and also reducing nitrates to nitrites in a marked degree, and he considers the stages of protein synthesis in the plant to be reduction of nitrate

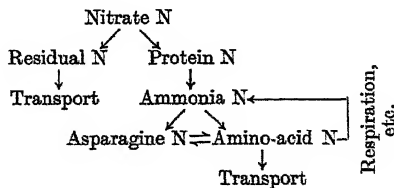
to nitrite. Nitrite now combines with formaldehyde to give formhydroxamic acid:



Formhydroxamic acid by further combination with sugars as well as higher alcohols gives rise to amino-acids, which subsequently condense to form proteins. The work of Baly, Heilbron and Hudson is based upon *in vitro* experiments and their deductions are highly theoretical, but they also, like Baudisch, assume that nitrate is first reduced to nitrite, and that subsequent combination between nitrite and formaldehyde leads to the production of formhydroxamic acid and eventually to amino-acids, proteins and nitrogen bases. The whole difficulty here is that up to the present time, in spite of repeated investigations, the presence of formaldehyde in normal leaves has not been shown with any degree of certainty. If the assumption be made that formhydroxamic acid is a stage of protein synthesis in the plant, however it may be formed, then it would condense *en bloc* to give protein and residual nitrogen. We would like to make it clear, at this stage, that with the evidence at present in our possession we are quite unable to state whether or no formhydroxamic acid does form such a stage in protein synthesis.

The nature of the fraction or fractions composing residual nitrogen is at present quite unknown. McKie(11), from her investigations of the protein metabolism of lupin seedlings, was able to obtain from 84 to 99 per cent. recovery of the total soluble nitrogen when she estimated proteose nitrogen. It is therefore possible that the residual nitrogen fraction is mainly composed of proteoses. From our results of the way in which residual nitrogen is formed, it would appear that this fraction is closely related chemically to protein.

Below we give a scheme summarising our results for the nitrogen metabolism of the healthy potato leaf:



VI. EXPERIMENTAL OBSERVATIONS ON LEAF-ROLL MATERIAL.

DIURNAL VARIATIONS IN LEAF-ROLL ARRAN VICTORY, SERIES I B
(MAY 16TH, 1932).(1) *Diurnal variations in total nitrogen of lamina and petiole.*

Fig. 10 shows the diurnal variations in total nitrogen of lamina and petiole of leaf-roll Arran Victory. The results have been calculated on a residual dry weight basis and are expressed in the same way as for healthy material.

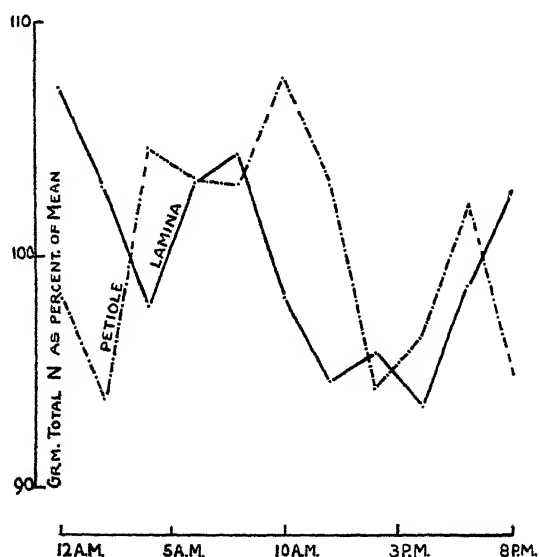


Fig. 10. Diurnal fluctuations in the total nitrogen content of laminae and petioles of leaf-roll Arran Victory. Results expressed per 100 gm. of residual dry weight. Plotted as a percentage of the mean value for each tissue.

The results again confirm Cockerham's findings. In the lamina the total nitrogen falls at first, rises to a maximum and then falls away continuously until 4 p.m., when it once more begins to mount. In the petiole, also, total nitrogen falls at first, but commences to rise before lamina nitrogen. After reaching its first maximum, it behaves irregularly, attains a second maximum and then falls sharply, and rises and falls again towards the close of the day. It is of interest to compare Fig. 10 with Fig. 1. The diurnal variation in total nitrogen which was so clear in the healthy lamina, with increase during the day and decrease during the night, is

absent in the leaf-roll lamina. After the first maximum is reached the curve falls continuously for several hours and then proceeds to mount towards the close of the day.

(2) *Diurnal variations in ammonia N, asparagine N, amino-acid N and nitrate N in the lamina (Arran Victory, Series Ib).*

The diurnal fluctuations in ammonia N, asparagine N, amino-acid N and nitrate N are graphically shown in Fig. 11. The first point of interest is the fact that the curves for ammonia N and amino-acid N follow the

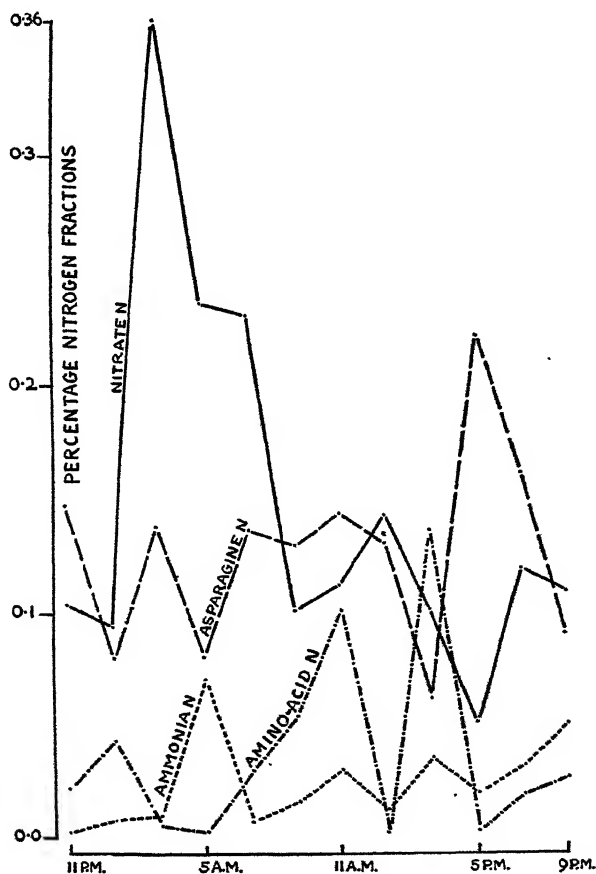


Fig. 11. Diurnal fluctuations in ammonia N, asparagine N, amino-acid N and nitrate N in the laminae of leaf-roll Arran Victory. Results expressed as a percentage of residual dry weight.

same course as in the healthy lamina (Fig. 3), but lag two hours behind in reaching the maximum and minimum points. The nitrate N curve shows accumulation at night and then falls away in the same manner as the curve for healthy material. In fact, the main difference between the behaviour of healthy and leaf-roll material is only to be found in the asparagine N curves. In the leaf-roll lamina asparagine N tends to fluctuate for the first few hours, but even here the two curves follow a very similar course after the initial fluctuations.

In Table VI we give the various direct correlation coefficients between these various fractions:

Table VI.

Ammonia/asparagine N	$r = -0.4185$
Ammonia/amino-acid N	$r = +0.0659$
Ammonia/nitrate N	$r = +0.0927$
Asparagine/amino-acid N	$r = -0.4974$
Asparagine/nitrate N	$r = -0.1009$
Amino-acid/nitrate N	$r = -0.3333$

None of these direct correlations are statistically significant, but that between ammonia N and asparagine N is high and is of the same sign as in the healthy lamina, and the correlation between ammonia N and amino-acid N is also high. The partial correlation coefficients are given in Table VII:

Table VII.

$C^r_{AB} = -0.4482$	$B^r_{AC} = -0.1805$	$B^r_{AD} = +0.0559$
$D^r_{AB} = -0.4129$	$D^r_{AC} = +0.1031$	$C^r_{AD} = +0.1219$
$CD^r_{AB} = -0.4213$	$BD^r_{AC} = -0.1739$	$BC^r_{AD} = -0.0551$
$A^r_{BC} = -0.5185$	$A^r_{BD} = -0.0687$	$A^r_{CD} = -0.3415$
$D^r_{BC} = -0.5661$	$C^r_{BD} = -0.3257$	$B^r_{CD} = -0.4438$
$AD^r_{BC} = -0.6728$	$AC^r_{BD} = -0.3056$	$AB^r_{CD} = -0.4419$

The corresponding regressions are given below:

$$A = -0.0126 - 0.2517B - 0.1038C - 0.0117D \quad \dots(1),$$

$$B = 0.0641 - 0.7086A - 0.6754C - 0.1156D \quad \dots(2),$$

$$C = -0.0783 - 0.2913A - 0.6702B - 0.1665D \quad \dots(3).$$

As in the healthy material, in none of the above equations does nitrate N play any significant part in the formation of the various fractions. Comparison with the equations given for the healthy leaf-blade is interesting in this case. It is clear that the formation of these various fractions in the leaf-roll lamina proceeds along the same lines as in the healthy lamina, and this is further borne out by calculating the significance of the difference of the means, using Fisher's table¹ for t .

¹ *Statistical Methods for Research Workers*, 1932.

Table VIII.

	$S(x-x')$	t	P
Ammonia N	0.0161	1.7840	0.1 -0.05 Not significant
Asparagine N	0.0723	2.5240	0.02-0.01 Significant
Amino-acid N	0.0066	0.4526	0.6 -0.5 Not significant
Nitrate N	0.0079	0.2265	0.8 -0.7 „

The only significant difference here is that between asparagine N in the healthy lamina and asparagine N in the leaf-roll lamina. It is doubtful, however, if this is of fundamental importance, because in leaf-roll President the difference of the means for asparagine N is not statistically significant, whereas the difference between ammonia N in the two cases is (see below).

(3) *Diurnal variations in protein N and residual N in the lamina and nitrate N in the petiole (Arran Victory, Series Ib).*

In Fig. 12 we give the diurnal variations in protein N and residual N in the lamina and nitrate N in the petiole. Nitrate N in the petiole shows a tendency to accumulation during the day, a feature absent in the healthy material. Comparison of Fig. 12 with Fig. 4 shows that in the latter protein N increases during the first eight hours, whereas in the leaf-roll lamina increase in protein N is of small duration (four hours). Comparison of residual N in the two cases exhibits no fundamental difference between the two curves.

We give in Table IX the various direct correlations between protein N, residual N and the other nitrogen fractions:

Table IX.

Protein/ammonia N	$r = -0.1978$
Protein/asparagine N	$r = -0.3381$
Protein/amino-acid N	$r = -0.2297$
Protein/nitrate N	$r = -0.0874$
Residual/ammonia N	$r = +0.1301$
Residual/asparagine N	$r = -0.2308$
Residual/amino-acid N	$r = +0.2382$
Residual/nitrate N	$r = -0.6368$
Residual/protein N	$r = +0.3620$

The correlation between protein N and nitrate N here is low and not statistically significant, whereas that between residual N and nitrate N is fully so. Thus, as far as the latter correlation is concerned, the results are the same as in the healthy lamina. It will be recalled, however, that the fluctuations of the nitrogen fractions in the leaf-roll lamina appeared to lag behind those in the healthy leaf-blade. If there be any significance in this lag period we should obtain an increased correlation with the

values shifted forward a period of two hours. When this is done for protein N and nitrate N, the correlation becomes fully significant ($r = -0.5671$). Here, again, protein formation falls into line with that

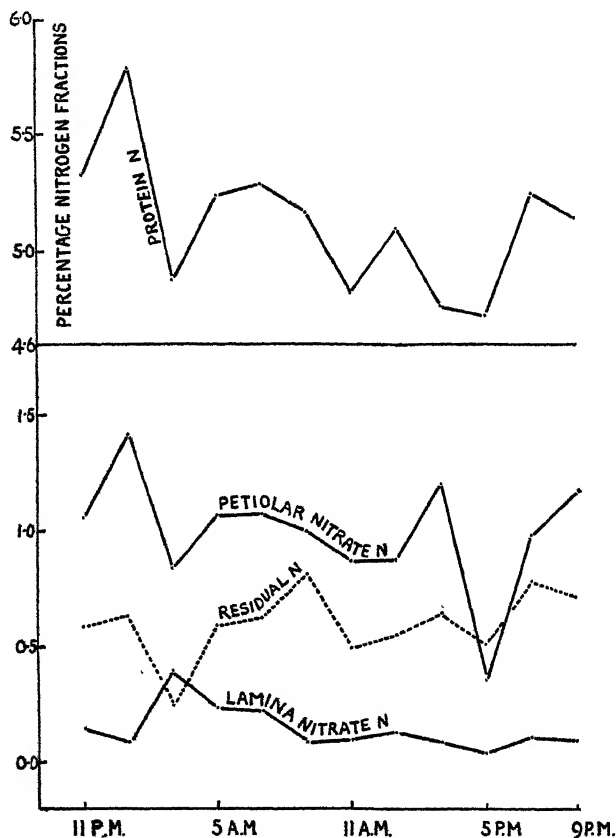


Fig. 12. Diurnal fluctuations in protein N, residual N and nitrate N in the laminae of leaf-roll Arran Victory, together with nitrate N in the diseased petioles. Results expressed as percentage of residual dry weight.

in the healthy leaf. We give the regressions for protein N, residual N and nitrate N below:

$$P = 4.348 + 0.8069D + 1.11R \quad \dots\dots(1),$$

$$R = 0.0147 - 0.9479D + 0.1432P \quad \dots\dots(2).$$

It is evident from these equations that both residual and protein N are coming from nitrate N, but that the bulk of the nitrate N is passing to

residual N and the latter is then converted to protein. In this conversion of residual N to protein N the leaf-roll lamina differs from the healthy blade.

When the significance of the difference of the means is obtained for residual N and protein N in healthy and diseased laminae, it will be seen that while the former is not statistically significant the latter is fully so:

Table X.

	$S(x - x')$	t	P	
Residual N	0.0958	1.676	0.2-0.1	Not significant
Protein N	0.6990	4.906	<0.01	Significant

DIURNAL VARIATIONS IN LEAF-ROLL PRESIDENT, SERIES IIb
(JULY 27TH, 1932).

(1) *Diurnal variations in total nitrogen of lamina and petiole.*

The curves for total nitrogen in lamina and petiole of leaf-roll President are shown in Fig. 13. The diurnal variation in total nitrogen, which is clearly shown by the curves for healthy lamina (Fig. 5), is not exhibited here. The curve falls during the greater part of the day and only mounts to a small extent towards the close of the experimental period. The petiolar curve is also falling during the greater part of the day and rising during the evening. We interpret these results as showing that synthesis of nitrogen compounds is not proceeding to the same extent as in healthy material, possibly due to the fact that there is accumulation of nitrate N in the petioles (see below) and that transport out of the laminae is also delayed.

(2) *Diurnal variations in ammonia N, asparagine N, amino-acid N and nitrate N in the lamina (President, Series IIb).*

Fig. 14 shows the diurnal fluctuations of ammonia N, asparagine N, amino-acid N and nitrate N in the leaf-roll laminae. The marked accumulation of nitrate N during the middle of the experimental period should be observed, as well as accumulation during the night. Asparagine N is at a maximum during the early part of the day and also towards the close of the day. Ammonia N in comparison with ammonia N in the healthy blade only slowly rises to a maximum.

Table XI gives the direct correlation coefficients between these various fractions.

Table XI.

Ammonia/asparagine N	$r = +0.1396$
Ammonia/amino-acid N	$r = +0.0312$
Ammonia/nitrate N	$r = -0.0462$
Asparagine/amino-acid N	$r = -0.6197$
Asparagine/nitrate N	$r = -0.0385$
Amino-acid/nitrate N	$r = -0.0331$

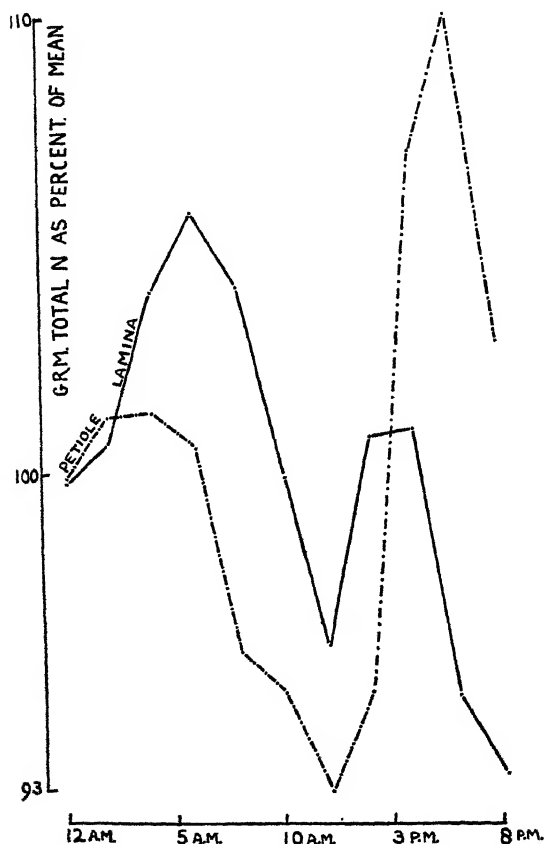


Fig. 13. Diurnal fluctuations in the total nitrogen content of laminae and petioles of leaf-roll President. Results expressed per 100 gm. of residual dry weight. Plotted as a percentage of the mean value for each tissue.

The only statistically significant correlation here is that between amino-acid N and asparagine N. It will be recalled that in Arran Victory leaf-roll the direct correlation coefficient between amino-acid N and asparagine N was high and became significant when the partial correlations

were calculated with elimination of ammonia N and nitrate N. There appears to be a particularly close relationship between asparagine N and

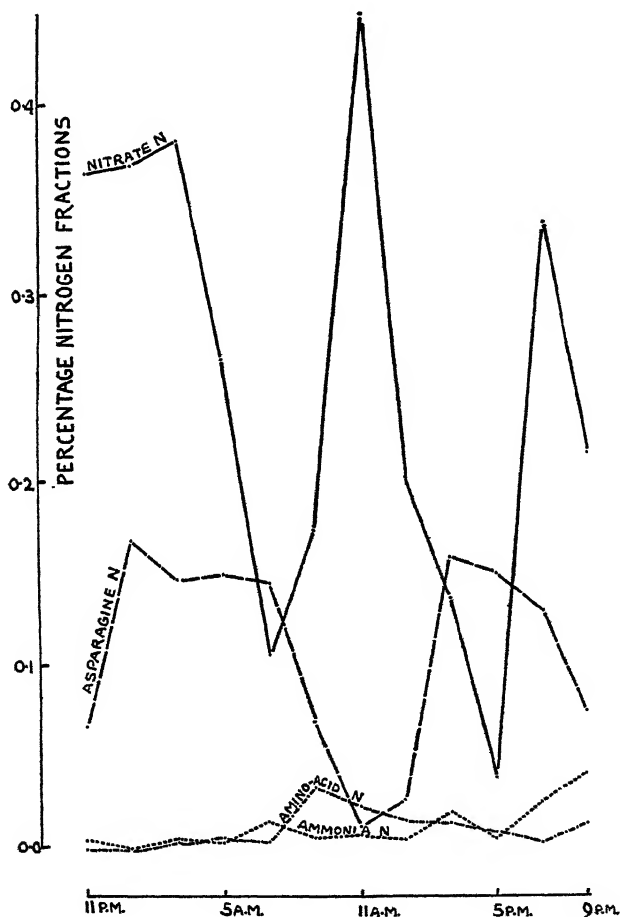


Fig. 14. Diurnal fluctuations in ammonia N, asparagine N, amino-acid N and nitrate N in the laminae of leaf-roll President. Results expressed as a percentage of the residual dry weight.

amino-acid N in leaf-roll material, possibly due to the fact that amino-acid N may form one of the fractions composing translocatory nitrogen and that, owing to the disturbance of the channels of transport, it is unable to escape from the lamina with the same facility as in healthy material.

We have calculated the necessary partial correlations and give the corresponding regressions below:

$$A = 0.0052 + 0.0486B + 0.2488C - 0.0023D \quad \dots\dots(1),$$

$$B = 0.1353 + 0.8826A - 4.355C - 0.0352D \quad \dots\dots(2),$$

$$C = 0.0189 + 0.0888A - 0.0918B - 0.0034D \quad \dots\dots(3).$$

If these equations be compared with those found for healthy material it will be seen that the formation of these fractions proceeds in the same way as in the normal leaf-blade. This is further borne out by the figures obtained for the significance of the differences of the means (Table XII):

Table XII.

	$S(x-x')$	t	P	
Ammonia N	0.0458	3.9670	<0.01	Significant
Asparagine N	0.0161	0.5887	0.6-0.5	Not significant
Amino-acid N	0.0153	1.9180	0.1-0.05	"
Nitrate N	0.0565	1.3600	0.2-0.1	"

In President ammonia N shows a statistically significant difference between healthy and leaf-roll lamina. It will be remembered that in Arran Victory, asparagine N showed a significant difference, but we do not attach any importance to either of these differences.

(3) *Diurnal variations in protein N and residual N in the lamina and nitrate N in the petiole (President, Series IIb).*

Fig. 15 gives the curves for protein N and residual N in the lamina and nitrate N in the petiole. As before we also give nitrate N in the lamina for sake of comparison. Nitrate N in the petiole shows marked accumulation during the major part of the day. Protein N is lower in amount than residual N. The latter rises markedly during the early part of the experimental period, falls away and rises once more at the close of the day. Protein N, on the other hand, after an initial fall rises slowly throughout the day and is at a maximum at the close of the experimental period. In Table XIII the direct correlation coefficients are given for residual N, protein N and the other nitrogen fractions:

Table XIII.

Protein/ammonia N	$r = -0.4052$
Protein/asparagine N	$r = -0.5333$
Protein/amino-acid N	$r = +0.2484$
Protein/nitrate N	$r = -0.0491$
Residual/ammonia N	$r = -0.3776$
Residual/asparagine N	$r = +0.5456$
Residual/amino-acid N	$r = -0.2254$
Residual/nitrate N	$r = -0.1949$
Residual/protein N	$r = -0.9396$

The correlation between protein N and asparagine N here is barely significant, and the correlation between residual N and asparagine N is also significant. The relation, however, between these fractions is through ammonia N, since in the partial correlations with ammonia N

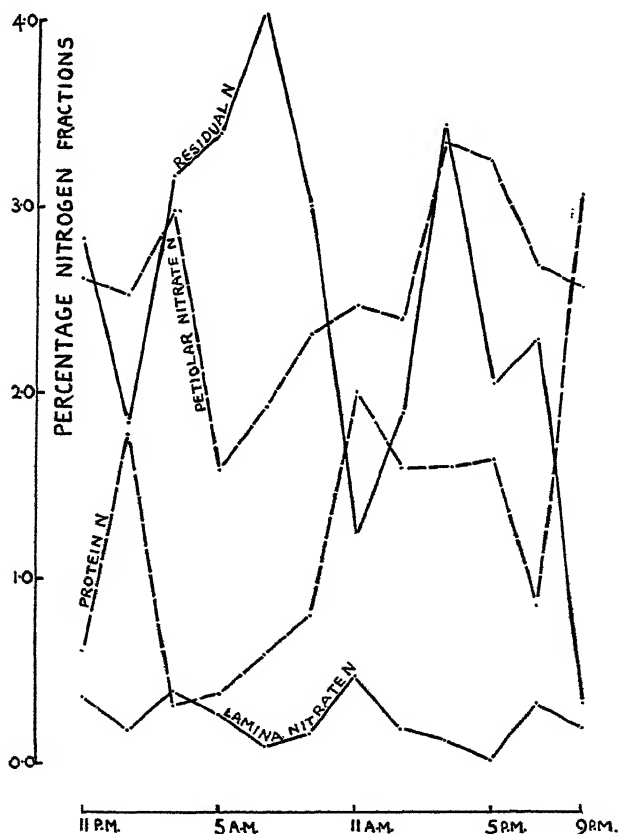


Fig. 15. Diurnal fluctuations in protein N, residual N and nitrate N in the laminae, together with nitrate N in the petioles of leaf-roll President. Results expressed as a percentage of the residual dry weight.

eliminated the values fall to non-significant figures ($r_{BP} = -0.5265$ and $r_{BE} = +0.5254$). The direct correlations between nitrate N, residual N and protein N are very low, but the partial correlations are statistically significant. For example, if residual N be eliminated from the protein/nitrate N coefficient, $r_{PD} = -0.6915$, which is fully significant,

and similarly with protein N eliminated from the residual/nitrate N correlation, ${}_P r_{RD} = -0.7047$, which is also fully significant. Thus, as in the case of Arran Victory, nitrate N passes directly to protein N and residual N. It will be recalled that it was shown that the formation of the various nitrogen fractions in Arran Victory appeared to lag a period of two hours behind in the leaf-roll lamina in comparison with the reactions taking place in the healthy blade, and in President healthy lamina that asparagine N formation appeared to lag behind ammonia N formation and that residual N and protein N appeared to lag two hours behind nitrate N. The question arises here, if there is a lag period of two hours in Arran Victory between healthy and diseased and a similar lag of two hours among the various nitrogen fractions of the healthy lamina of President, then the values for diseased President should lag still further behind those in the healthy blade. If a shift on of four hours be made in the President leaf-roll values, the correlation between residual N and nitrate N becomes $r = +0.9559$, and between protein N and nitrate N, $r = -0.8696$. Both values are statistically significant, and add confirmation to the view that nitrate N is proceeding directly to form residual N and protein N.

The direct correlation between residual N and protein N in this instance is also statistically significant and very high, $r = -0.9396$, and the partial correlation with nitrate eliminated becomes ${}_D r_{PR} = -0.9390$. It is evident that the reaction between these two fractions is proceeding independently of the presence of the other fractions. The regressions between protein N, residual N and nitrate N are given below:

$$R = 4.301 - 1.171P - 1.949D \quad \dots\dots(1),$$

$$P = 3.523 - 0.8017R - 1.582D \quad \dots\dots(2).$$

These again give confirmation that nitrate N is proceeding to protein and residual N and that at the same time there is a reversible reaction between residual N and protein N (*vide* Arran Victory, Series Ib).

The significance of the difference of the means for residual and protein N in healthy and leaf-roll lamina are given in Table XIV:

Table XIV.

	$S(x-x')$	t	P	
Residual N	0.0620	0.1986	0.9-0.8	Not significant
Protein N	2.2440	8.3640	<0.01	Significant

As in Arran Victory, so in President, residual N in the two cases does not differ significantly, but protein N does.

VII. SEASONAL VARIATIONS.

In Figs. 16 and 17 we have plotted the seasonal variations of protein N, residual N and nitrate N in Arran Victory and President leaf-roll

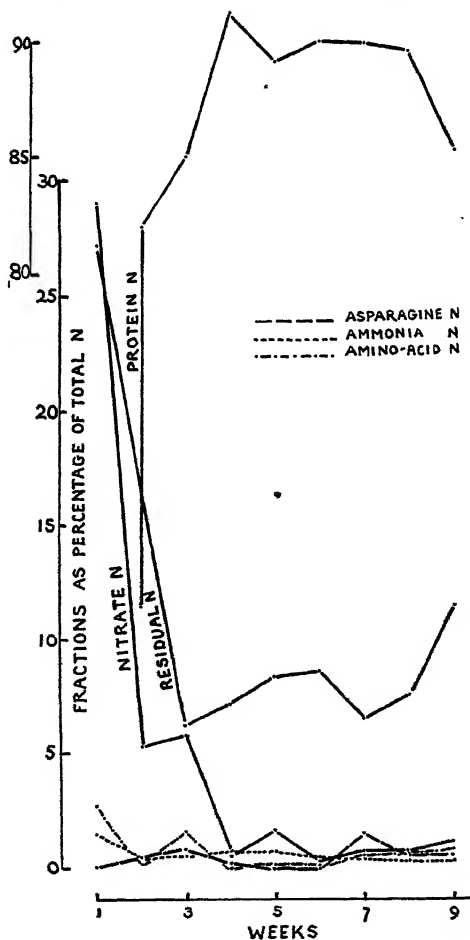


Fig. 16. Seasonal fluctuations in protein N, residual N, nitrate N, ammonia N, asparagine N and amino-acid N in the laminae of leaf-roll Arran Victory. Samples gathered at weekly intervals and results expressed as a percentage of the total nitrogen.

laminae. We have also included in Fig. 16 (Arran Victory) the fluctuations in ammonia N, asparagine N and amino-acid N.

Considering Fig. 16 first, there is a heavy fall in nitrate N over the first three weeks, while protein N shows a corresponding rise over this

time. We again interpret this as showing synthesis of protein N from nitrate N and also that the main amount of protein synthesis takes place

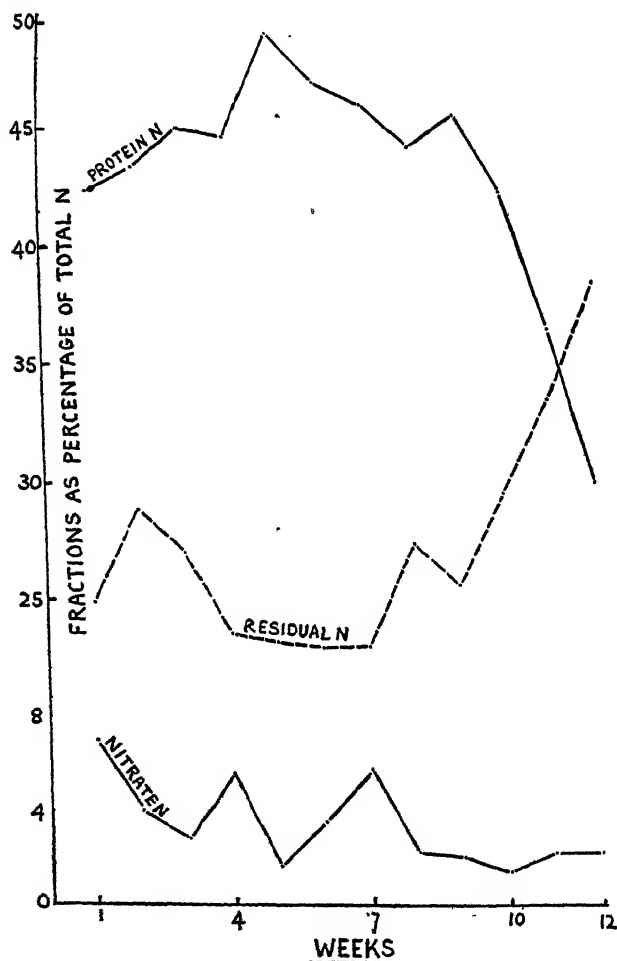


Fig. 17. Seasonal fluctuations in protein N, residual N and nitrate N in the laminae of leaf-roll President. Samples gathered at weekly intervals and results expressed as a percentage of total nitrogen.

in the first weeks of growth. Comparison of the curve for residual N in healthy material (Fig. 8) with that in leaf-roll is of interest. Residual N falls at first, due possibly to its synthesis to protein (see Section VI, (3), p. 575) and thereafter rises until it reaches a maximum at the close of the

growing season. We have already stated, following the interpretation of Maskell and Mason, that residual N probably forms the necessary head for soluble nitrogen prior to transport down the stem. This view appears to us to be confirmed by the present curves. In leaf-roll the phloem suffers disruption and translocation is hindered, and the accumulation of residual N in the diseased laminae towards the end of the season seems to point to the fact that it cannot escape from the lamina with the same ease as in healthy material. A further fact that seems to us to confirm this view is the interconversion that we were able to show in leaf-roll laminae of residual N and protein N; a reaction which is absent in the healthy laminae.

Fig. 17 shows the seasonal fluctuations for protein N, residual N and nitrate N in the diseased leaves of President. We have omitted the values for ammonia N, asparagine N and amino-acid N, since these, as in Arran Victory, were very small in amount. The marked accumulation of residual N should be noticed, and also that nitrate N falls over the first few weeks while protein N rises.

VIII. TRANSLOCATION.

The question of translocation of nitrogenous compounds has proved a difficult subject to solve, and Maskell and Mason (see above), after an extensive series of investigations, were only able to suggest that possibly residual N forms the necessary head in the lamina for transport and that all soluble nitrogen fractions, including soluble protein, can take part in longitudinal passage down the stem. We are largely in agreement with this view that residual N forms the necessary head in the leaf-blade. It will be recalled that our seasonal analyses showed marked accumulation of residual N in the diseased laminae. Since in leaf-roll the main channel of transport of elaborated food material (the phloem) is disturbed and in certain cases even disrupted, and if residual N does form the necessary head for transport, this result is to be expected. We are unable to suggest whether or no all soluble nitrogen compounds take part in longitudinal transport as was suggested by Maskell and Mason. We were unable to find any statistically significant correlations between lamina residual N and petiolar residual N in either Arran Victory or President. On the other hand, the simple regression between lamina residual N and petiolar residual N shows that the direction of transport is from lamina to petiole. Calling lamina residual N, X , and petiolar residual N, Y , the regression becomes:

$$X = 0.4351 + 0.1305Y \quad \dots(1),$$

$$Y = 0.2168 + 0.7461X \quad \dots(2).$$

That residual N probably plays an important part in longitudinal transport is further borne out by the fact that in leaf-roll Arran Victory petioles it is very small in amount, while in President, which is attacked by the disease to a far greater extent, it is entirely absent, and the sum of the ammonia N, asparagine N, amino-acid N and nitrate N was equal to the total soluble N. We have initiated a further series of investigations on this matter in connection with further work on nitrogen metabolism of other virus diseases, and we hope that it will be possible to give a more definite answer to this question later.

IX. TUBER ANALYSES.

We have made a number of analyses of the nitrogen fractions of healthy and diseased tubers. It was found by Cockerham that there was a gradient of total N in both healthy and leaf-roll tubers from eye to cortex and cortex to medulla. We have been able to confirm these results and give below the gradients of the various nitrogen fractions in these three regions of the tuber for healthy and leaf-roll President:

Table XV.

President. (Healthy tuber. Results expressed as a percentage of total dry weight.)

	Ammonia N	Asparagine N	Nitrate N	Residual N	Protein N
Eye	0.1660	0.1107	0.0553	0.3595	1.5280
Cortex	0.0721	0.0521	0.1043	0.2147	1.1180
Medulla	0.1081	0.1082	0.1352	0.1353	1.0100

Table XVI.

President. (Leaf-roll tuber. Results expressed as a percentage of total dry weight.)

	Ammonia N	Asparagine N	Nitrate N	Residual N	Protein N
Eye	0.2309	0.1890	0.1386	0.4111	1.7490
Cortex	0.1006	0.1006	0.1758	0.2540	1.7240
Medulla	0.1273	0.1546	0.1781	0.1508	0.9910

We have not thought it necessary to give the results for Arran Victory, as these were essentially the same as for President, and we have used the President results here as this variety is particularly intolerant of leaf-roll. It will be seen that there is a negative gradient of nitrate N in both healthy and diseased tubers from eye to medulla. Residual N in both cases shows a positive gradient from eye to medulla, and the same applies to protein N. The gradient of protein N in leaf-roll from medulla to cortex is slightly steeper than in the healthy material. Schweizer (21) claimed

that in leaf-roll plants there is a migration of protein to the developing shoot which leads to a complete cessation of diastatic activity in the tuber, with the result that sugars are prevented from passing to the developing aerial portions. This suggestion is probably correct. It will be remembered that in our seasonal analyses there was a marked increase of protein N in the leaf-roll lamina in the early weeks of growth, an increase that was especially prominent in President (see Fig. 17), and it is possible that part of this protein N may have been rapidly migrated from the tuber.

X. GENERAL DISCUSSION.

It is clear if our results be taken as a whole that there are not the same marked differences between healthy and leaf-roll plants in their nitrogen relations as was found by us for their carbohydrate metabolism. It is also evident that leaf-roll is not a disease due to protein derangement, as was claimed by Schweizer. There is a significant difference between protein in the healthy leaf and protein in the diseased blade in both Arran Victory and President it is true, but, on the other hand, the method of formation of protein and the other nitrogen fractions was shown by us to proceed along the same lines in the two cases, although in the leaf-roll plants the reactions appear to lag behind the healthy.

On our view that protein N and residual N are directly formed from nitrate N, this result can be explained. We showed in our previous communication(3) on the carbohydrate metabolism of leaf-roll plants that photosynthesis was either absent or very small in amount. Assuming that formaldehyde is the first stage in photosynthesis, although we are entirely without evidence on this point, and further assuming that formhydroxamic acid is a stage in protein synthesis, if photosynthesis be small or absent in leaf-roll plants, combination with nitrate N cannot take place to the same extent as in healthy material, and this would be one cause of reduction in protein. There is also the fact to be considered that nitrate N shows accumulation in leaf-roll petioles, as though it cannot pass into the diseased laminae with the same facility as in the healthy. This fact also would account for reduction in protein in leaf-roll plants.

XI. SUMMARY.

The present investigation is concerned with the differences between the nitrogen metabolism of healthy and leaf-roll affected potato plants.

Diurnal estimations were made of the total nitrogen as well as the following nitrogen fractions, ammonia N, asparagine N, amino-acid N,

nitrate N, residual N and protein N in the leaves and petioles of healthy and leaf-roll infected Arran Victory and President. Weekly determinations of total nitrogen and the above nitrogen fractions were also made in the laminae of Arran Victory and President throughout the growing season.

Evidence is presented to show that there is apparently no fundamental difference in the nitrogen metabolism of healthy and leaf-roll plants, and that the formation of nitrogenous compounds proceeds along the same lines in the two cases.

A theory is put forward to account for protein synthesis direct from nitrate N, and not via amino-acids. The problem of translocation is also discussed, and it is considered that the residual N fraction plays an important part in nitrogen transport.

We wish to express our indebtedness to Mr William Robb, F.R.S.E., for reading the manuscript and making a number of suggestions. We would also like to take this opportunity of thanking Dr W. H. Pearsall, of the Botany Department, The University, Leeds, for his help and assistance in a number of different directions. This work was carried out at the Station of the Scottish Society for Research in Plant Breeding, Corstorphine, Edinburgh, under a scheme supported by a grant from the Empire Marketing Board.

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THE INFLUENCE OF ENVIRONMENTAL CONDITIONS ON THE DEVELOPMENT OF THE ANGULAR LEAF-SPOT DISEASE OF COTTON

V. THE INFLUENCE OF ALTERNATING AND VARYING CONDITIONS ON INFECTION

By R. H. STOUGHTON, D.Sc.

(*Department of Plant Pathology, Rothamsted Experimental
Station, Harpenden.*)

INTRODUCTORY.

IN earlier papers of this series the influence of soil temperature(3), air temperature(4) and atmospheric humidity(5), in conditioning the attack of young cotton plants by the bacterial parasite, *Bacterium malvacearum*, were considered. In all the experiments so far described the environmental factors under consideration were maintained constant throughout each experiment, in an attempt to disentangle the individual effects. In the experiments to be outlined in this paper the effect has been studied of varying one or more of the factors either in a uniform manner or by an abrupt transition from one value to another at different periods during the incubational period of the disease. In some of the experiments a regular diurnal variation has been arranged, and such conditions approximate more closely to those occurring in nature than has been the case in any of the experiments previously described. It is clear that experiments on the influence of varying conditions cannot be interpreted save in the light of similar tests carried out with the conditions maintained constant, but when the results of the latter are available the effect of fluctuating conditions may throw useful light on the problem of the influence of the environment in nature.

These experiments have all been carried out in the special Rothamsted control chambers erected and operated under a grant from the Empire Marketing Board(2). The seed used throughout the work has been the "Sakellarides" variety from the Sudan, supplied by the courtesy of Mr R. E. Massey, Botanist to the Sudan Government.

THE INFLUENCE OF SOIL TEMPERATURE VARIATIONS.

Regular diurnal variation.

Exp. 1. In order to provide for an even diurnal variation of soil temperature some modifications in the standard controls of one chamber were necessary. The water from the soil temperature tank was drained out and the tank dried. A length of rubber tubing connected to an electric blower was passed through the water-inlet tube and brought down inside the tank, the open end being turned upwards and held in a retort clamp standing on the floor of the tank. When the soil tins were in place, the air blown into the tank escaped through the open water-outlet tube. The mercury thermostat was removed and the hole in the cover plugged. The relay for soil temperature control was removed from the electrical circuit to the tank heaters and in its place was substituted a small automatic time-switch. Two of the three heaters were disconnected, and the time-switch was set to cut in the supply to the remaining heater at 12 noon, and to cut out at 12 midnight. By adjustment of the air stream through the tank it was possible to obtain a gradual rise in the temperature of the soil in the tins for 12 hours, followed by a gradual fall to the original temperature. This fluctuation remained very regular and constant throughout the experiment, ranging from 25 to 37° C. Three of the remaining chambers were used with the standard controls, and the soil temperature thermostats were set to give temperatures of 25–26° C., 30–31° C., and 37–38° C., respectively. The air temperature thermostats in all four chambers were set for a constant temperature of 27° C. Air humidity was not controlled in these experiments, but exceeded 80 per cent. in all chambers except during short periods in the chamber arranged for the alternating soil temperature.

Before sowing, the seed was soaked in a strong emulsion of a virulent culture of *B. malvacearum*, and then sown at the rate of ten seeds per tin, giving a total of eighty seeds in each chamber. The seedlings appeared in 2 days at the higher temperature and in 3 to 4 at the lower. When the seedlings appeared above the soil, the artificial illumination was turned on, and the lighting control time-switch set for a lighting period of 14 hours, from 6 p.m. to 8 a.m. Thus the period of maximum soil temperature coincided with the time of illumination.

Infection spots on the cotyledons were visible very soon after germination. The spots were in most cases very small on the seedlings at the two higher soil temperatures, but were larger at the soil temperature of 25° C. The cotyledons were fully developed and infection appeared to be

complete 12 days after sowing, and the seedlings were then carefully examined for infection. The results are given in Table I, where the total number of seedlings in each tin, the number infected, the average germination and the average percentage infection are given.

Table I.

Infection at the four different soil temperatures. Exp. 1.

Tin	25-26° C.		30-31° C.		37-38° C.		Alternating 25-37° C.	
	No. of seedlings	No. in- fected	No. of seedlings	No. in- fected	No. of seedlings	No. in- fected	No. of seedlings	No. in- fected
I	7	0	6	2	4	1	8	1
II	7	4	8	2	7	0	7	1
III	7	1	8	1	7	1	6	1
IV	7	1	8	4	7	0	9	1
V	9	5	8	2	7	0	7	4
VI	9	4	8	1	5	2	6	2
VII	8	3	7	1	3	0	5	0
VIII	10	4	Accidentally destroyed		6	1	9	1
Total	64	22	53	13	46	5	57	11
%	80	34.4	76.4	22.6	57	10.9	71	19.3

It will be seen that the percentage infection decreases with increase of constant soil temperature, as was found in the earlier experiments on this factor (3). The amount of infection under the condition of a regular daily variation in soil temperature is somewhat less than at a constant temperature equal to the mean of the variation, *i.e.* 30-31° C., but the difference is hardly significant.

Exp. 2. This experiment was in all respects an exact repetition of the previous one, carried out under the same conditions. The results are given in Table II.

Table II.

Infection at the four different soil temperatures. Exp. 2.

Tin	25-26° C.		30-31° C.		37-38° C.		Alternating 25-37° C.	
	No. of seedlings	No. in- fected	No. of seedlings	No. in- fected	No. of seedlings	No. in- fected	No. of seedlings	No. in- fected
I	12	0	12	0	9	0	11	1
II	12	3	12	1	9	0	9	0
III	12	0	9	1	10	0	10	0
IV	11	3	11	1	10	0	11	0
V	12	3	12	2	10	0	10	1
VI	12	2	11	1	8	0	11	0
VII	12	1	12	2	8	0	11	2
VIII	11	1	12	0	10	0	11	0
Total	94	13	91	8	74	0	84	4
%	98	13.8	95	8.8	77	0	87	4.8

The general level of infection was lower than in Exp. 1, probably owing to a lesser degree of virulence of the culture used, but the order of the results is precisely as in the first experiment. It may be concluded, then, from these two trials that the effect of a regular diurnal variation of soil temperature on infection under these conditions is approximately equivalent to that of a constant temperature near to, or slightly above, the mean of the fluctuating temperature.

Abrupt change.

Having established the effect of a regular daily variation in soil temperature it remained to investigate the time factor in the influence of the temperature of the soil, that is to say, whether the temperature at the time of germination, or the temperature after germination has occurred, is the determining factor in controlling the amount of primary infection. To investigate this an experiment was carried out in which the soil temperature was altered at a definite time after sowing, and thereafter maintained constant at the changed temperature. At the same time an attempt was made to study the effect of soil moisture and of different types of soil on the primary infection. Two very different soils were used in this experiment. One was the Gezira cotton soil, used in all previous experiments, while the other was an old turf loam which had been used for tomato culture in the glasshouse.

Exp. 3. A sufficient quantity of each soil to fill twenty-four of the soil tins was prepared by spreading on a concrete floor to dry to a moderately low moisture content. Each batch of soil was then thoroughly mixed to ensure a uniform moisture content and twenty-four tins filled with each soil. Four tins of each soil were then placed in the control chambers, the tins being arranged alternately to allow for any unevenness in the distribution of light from the electric lamps. Two tins of each soil in each chamber were then watered with 1000 c.c. of tap water and two with 400 c.c. to give two different moisture contents. Each treatment was thus duplicated in each chamber. Samples of soil from each tin were taken after 2 days and the moisture contents determined. These gave average values of 26 and 23 per cent. for the "wet" and "dry" Gezira soils respectively, and 32 and 29.5 per cent. for the glasshouse soils. An amount of water was then added to each tin calculated to bring the moisture contents approximately to 30 and 23 per cent. for the Gezira, and 35 and 29.5 per cent. for the glasshouse soils. By this treatment the "wet" soils were nearly saturated and the "dry" were in a good condition for normal growth though rather on the dry side. After adjustment of the soil

temperatures to the values to be referred to later, the tins were sown at the rate of twenty seeds per tin with cotton seed from the normal Sudan crop of 1931-2. Before sowing, the seed was soaked in a very strong emulsion of *B. malvacearum* in water under the vacuum pump and then spread out overnight in the laboratory to become nearly dry.

The soil temperature in the chambers at the time of sowing and the subsequent changes were as follows:

Chamber 1.	20° C.,	constant.		
„	2.	20° C.,	raised to 33° C.	2 days after sowing.
„	3.	20° C.,	„	3 „
„	4.	33° C.,	constant.	
„	5.	33° C.,	reduced to 20° C.	2 days after sowing.
„	6.	33° C.,	„	3 „

The air temperature in all chambers was controlled at 32-33° C. The humidity was uncontrolled but was high in all chambers, though naturally lower in the chambers with a low soil temperature.

During growth, water was added from time to time in amounts thought to be necessary to maintain the moisture contents of the soils. This was, of course, largely a matter of guesswork, and in fact rather too much water was added, so that in most cases the moisture content rose during the experiment. At the close of the experiment the moisture content of each tin was again determined. The results of this determination are shown in Table III.

It will be seen that while the average values for the Gezira soils were maintained fairly well, the glasshouse soils were decidedly wetter at the end of the experiment than at the beginning. The differences between the "wet" and the "dry" soils was, however, retained throughout.

Germination was, unfortunately, poor, less than half the seeds sown producing plants. The experiment was allowed to run for two weeks, and at the end of this time the seedlings were examined for primary infection. The infection was not severe, but in accordance with the general rule for these experiments the presence of a single infection-spot on a cotyledon was counted as an infected plant. The results of the examination are shown in Table IV, which gives the total number of plants in each tin, the number infected, the percentage germination, and the percentage infection for each soil temperature condition and each type of soil.

Considering first the influence of soil temperature, a comparison of the total percentage infection in Chamber 1 (20° C., constant) with that in Chamber 4 (33° C., constant) shows the expected effect of reduction

Table III.
Moisture contents of ins. Exp. 3.

Soil	Initial moisture content All chambers %	Final moisture content						Mean %
		Chamber 1 %	Chamber 2 %	Chamber 3 %	Chamber 4 %	Chamber 5 %	Chamber 6 %	
Gezira "Wet" I	30	30.2	32.0	33.2	27.9	31.4	31.7	30.6
" " II	30	29.8	28.5	33.9	26.9	29.8	31.5	31.5
Glasshouse " " I	35	40.9	41.2	44.9	38.7	45.8	46.2	42.7
" " II	35	38.3	41.6	45.0	36.6	47.2	46.1	42.7
Gezira "Dry" I	23	25.3	25.8	25.2	23.6	24.2	20.1	24.0
" " II	23	25.6	23.3	24.0	23.7	23.9	22.8	23.8
Glasshouse " " I	29.5	35.8	37.2	34.3	32.1	31.6	33.2	34.3
" " II	29.5	33.6	35.5	36.8	32.7	34.0	34.4	34.3

Table IV.

Infection at different soil temperature conditions and soil moistures. Exp. 3.

Soil	No. of plants infected	Chamber 1		Chamber 2		Chamber 3		Chamber 4		Chamber 5		Chamber 6		Mean germination %	Mean infection %
		No. of plants infected	No. of plants infected	No. of plants infected	No. of plants infected	No. of plants infected	No. of plants infected	No. of plants infected	No. of plants infected	No. of plants infected	No. of plants infected	No. of plants infected	No. of plants infected		
Gezira "Wet" I	12	5	10	7	8	5	10	1	7	1	8	0	41.2	29.4	
" " II	8	3	5	2	5	2	6	0	13	2	7	1	40.4	19.6	
Glasshouse " " I	7	3	8	1	6	1	10	1	15	1	9	0	40.0	21.9	
" " II	13	6	6	3	4	2	4	0	6	0	9	2	45.0	14.8	
Gezira "Dry" I	7	2	9	2	6	0	7	1	10	3	9	2			
" " II	7	3	10	3	10	2	5	0	6	1	10	2			
Glasshouse " " I	10	0	10	0	10	1	8	0	11	2	9	1			
" " II	12	3	6	0	4	2	10	2	8	2	10	1			
Total	76	25	64	18	53	15	60	5	76	12	71	9			
Mean (%)	46.5	32.9	40.0	28.2	33.1	28.3	37.5	8.3	47.5	15.8	44.3	12.7			

in infection with high temperatures. The importance of the time at which the high temperature prevails is, however, clearly shown by a comparison of the figures for the other chambers. Where the germinating seeds were exposed to a low temperature for the first 2, or 3, days after sowing (Chambers 2 and 3) a subsequent raising of the temperature after this period has not appreciably reduced the amount of infection compared with those exposed to a low temperature throughout. On the other hand, a high temperature at the time of sowing and for the following 2, or 3, days (Chambers 5 and 6) reduces the infection very greatly, in spite of the subsequent reduction in temperature. The differences in infection between Chambers 4, 5 and 6 are of doubtful significance, although there are indications that the reduction in temperature 2 days after sowing has allowed some increase in infection over the controls to occur. The experiment shows clearly that the soil temperature is of chief importance at the time of sowing and during the first few days of germination, a high temperature at this period causing a considerable reduction in the amount of infection, while subsequent variations of temperature exert little or no effect.

Turning now to the influence of type of soil and moisture content, the figures given in the last two columns of Table IV show some interesting results. In the first place it is apparent that neither the type of soil nor the moisture content have had any appreciable effect on the total germination. The seeds in the "wet" series germinated more quickly than those in the "dry," but the final numbers are approximately the same in all series. On the other hand, both soil type and moisture content appear to have exercised some influence on the amount of infection. The mean percentage infection for the Gezira soil is definitely greater than for the glasshouse loam, while within the soil type a high moisture content is associated with a higher degree of infection than occurs with a more normal amount of water. The differences observed in the latter case, namely 7 and 10 per cent., are not high, but the fact that they are in the same direction in both soils makes the assumption of a definite influence of moisture content of the soil a reasonable one. The argument is strengthened by an examination of the figures for the separate chambers, the highest infections of the whole experiment occurring in the "wet" Gezira soils at a low soil temperature (Chambers 1, 2 and 3), where the mean percentage infections for duplicates are 40, 60 and 54 per cent. respectively.

THE INFLUENCE OF AIR TEMPERATURE VARIATIONS.

Regular diurnal variation.

To provide for a regular daily variation of air temperature a special type of thermostat was designed and constructed by Messrs Venner Time Switches, Ltd., of London. Briefly, this instrument consists of a direct-acting thermostat controlling the temperature, while the setting of the instrument is continually altered by a circular cam, driven by a clock, and revolving once in each 24 hours. The cam can be designed to give any desired range of temperature, while provision is made for setting the instrument to any required mean temperature. In the instrument used the cam was designed to give a range of 10° C. on either side of the mean. This apparatus replaced the standard thermostat and relay in the electrical circuit to the air-chamber heating coils.

Exp. 4. The results of part of this experiment were given in an earlier paper of this series(4), which dealt with the effect of constant air temperatures, but they must be recapitulated here for comparison with the remainder of the experiment, which concerned the influence of alternating conditions. The forty-eight tins for the six chambers were filled with cotton soil imported from the Gezira, and sown, in the glasshouse, with four seeds per tin. When of a suitable size the plants were transferred to the chambers for a few days and then sprayed with a strong emulsion of the bacteria. Five of the chambers were arranged to give constant air temperatures of 39, 35, 30, 25, 23.5° C. respectively, while the sixth was provided with the special alternating thermostat set to give a daily range from $20-40^{\circ}$ C. Owing to the heating effect of the soil, which was kept at a constant temperature of $27-28^{\circ}$ C. in all chambers, the minimum temperature reached each day in this chamber was about 22° C., while the maximum attained was usually about 39° C. Thus the alternation covered the full range of temperatures provided in the other five chambers. The relative humidity in all chambers was controlled between 80-85 per cent. Half of the total number of plants in each chamber were sprayed in the dark and half in the light. Artificial illumination was provided for 16 hours daily, the times of illumination coinciding with the high temperature periods in the alternating temperature chamber. The approximate incubation periods for the disease at the different temperatures, that is, the time from inoculation to the appearance of visible symptoms, are given in Table V. The plants at 39° C. constant temperature made no growth and many were dying at the close of the experiment. No definite

infection was detectable on the leaves of these plants, and they are omitted in the further discussion.

Table V.

Incubation periods at the different air temperatures. Exp. 4.

Temperature	35° C.	30° C.	25° C.	23.5° C.	Alternating 22–39° C.
Incubation period (days)	6	7–8	12	14	11–12

The plants at 35° C. were examined after 21 days and the remainder 3 days later. The method adopted for estimating the incidence and degree of infection was considered in detail in an earlier paper(4). Each leaf was examined, the number of spots counted on the standard basis, and the results grouped into four classes: Class I, severe infection, fifty spots or more per leaf; Class II, moderate infection, twenty-five spots or more; Class III, light infection, ten spots or more; Class IV, very light infection, less than ten spots. The results are summarised in Table VI.

The figures relating to constant temperatures were considered in the previous paper, and we are concerned here only with the influence of the alternating temperature. As in the case of a regular diurnal variation of soil temperature, the effect of a similarly fluctuating air temperature is to result in a degree of infection approximately equivalent to that obtained at a constant temperature near to the mean of the alternation. The infection is somewhat less than at a constant temperature of 30° C. but decidedly higher than at 25° C. Consideration of the effect of light and time of spraying in relation to the period of illumination will be deferred until later in this paper. A second trial of the influence of a regular alternation of temperature was incorporated in the experiment next to be described, which concerned mainly the effect of abrupt changes in the air temperature during the incubation period.

THE INFLUENCE OF ABRUPT CHANGES IN AIR TEMPERATURE.

Exp. 5. This experiment was designed to test whether the determining factor in infection with regard to air temperature is the actual temperature at the time of inoculation or the mean temperature prevailing during the incubation period.

Gezira soil was used, and plants were raised in the soil tins in the glass-house. When the plants were about six weeks old they were transferred to the chambers for a few days before spraying. The soil temperature thermostats were set for a temperature of 30° C. and the humidity controls for a relative humidity of 85–90 per cent. The following plan of air temperature changes was carried out.

Table VI.

Distribution of infection in four classes at various air temperatures, Exp. 4.

	35° C.				30° C.				25° C.				23.5° C.				Alternating 22-39° C.			
	Dark		Light		Dark		Light		Dark		Light		Dark		Light		Dark		Light	
	No. of leaves	%	No. of leaves	%	No. of leaves	%	No. of leaves	%	No. of leaves	%	No. of leaves	%	No. of leaves	%	No. of leaves	%	No. of leaves	%	No. of leaves	%
Total no. of leaves	78	—	78	—	68	—	62	—	59	—	55	—	64	—	60	—	58	—	63	—
Class I, 50 spots and over	16	20.5	1	1.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1.6
" II, 25 "	3	3.9	1	1.3	2	2.9	1	1.6	0	0	0	0	0	0	0	0	0	0	2	3.2
" III, 10 "	7	8.7	2	2.6	6	8.8	3	4.8	1	1.7	0	0	0	0	0	0	1	1.7	0	0
" IV, less than 10 spots	26	33.4	16	20.5	23	33.9	11	17.8	5	8.5	4	7.3	4	6.3	3	5.0	9	15.5	12	19.1
Total no. of leaves infected	52	66.5	20	25.7	31	45.6	15	24.2	6	10.2	4	7.3	4	6.3	3	5.0	10	17.2	15	23.9
Av. no. of spots per leaf	18.70		2.32		4.71		1.60		0.61		0.18		0.17		0.07		0.78		2.13	

Table VII.

Distribution of infection in four classes at the various air temperature conditions, Exp. 5.

	Chamber 1		Chamber 2		Chamber 3		Chamber 4		Chamber 5		Chamber 6	
	No. of leaves		No. of leaves		No. of leaves		No. of leaves		No. of leaves		No. of leaves	
	%		%		%		%		%		%	
Total no. of leaves	142	—	98	—	132	—	154	—	129	—	178	—
Class I, 50 spots and over	1	0.7	0	0.0	6	4.5	7	4.5	3	2.3	18	10.1
" II, 25 "	1	0.7	2	2.0	1	0.8	7	4.5	2	1.6	10	5.6
" III, 10 "	6	4.2	5	5.1	15	11.4	9	5.8	10	7.8	11	6.2
" IV, less than 10 spots	27	19.0	9	9.2	18	13.6	18	11.7	32	24.8	38	21.4
Total no. of leaves infected	35	24.6	16	16.3	40	30.3	41	26.5	47	36.5	77	43.3
Av. no. of spots per leaf	2.05		1.71		5.48		6.01		4.05		10.62	

Chamber 1. Temperature at time of spraying 34° C., maintained constant for 24 hours, then reduced to 22° C. and thereafter kept at this temperature.

Chamber 2. As Chamber 1, but 34° C. maintained for 48 hours after spraying.

Chamber 3. Temperature at time of spraying 22° C., maintained constant for 24 hours, then raised to 34° C. and thereafter kept at this temperature.

Chamber 4. As Chamber 3, but 22° C. maintained for 48 hours after spraying.

Chamber 5. Regular diurnal alternation 22–35° C.

Chamber 6. Constant temperature of 34–35° C.

As has always been the case in working at comparatively low air temperatures it proved impossible to prevent a small rise in air temperature when the lights were on, and where the figure of 22° C. is given it should be noted that this represents the mean temperature during the 24 hours, the maximum range being 4° C., *i.e.* from 20 to 24° C. This difficulty did not arise with the higher temperature, and here the total range of variation did not exceed 1° C.

Infection was visible in 5–6 days in Chambers 5 and 6, in 6–7 days in Chambers 3 and 4, and in 7–8 days in Chambers 1 and 2. The plants were examined and the degree of infection estimated the following number of days after spraying: Chamber 6, 14 days, Chambers 5 and 3, 15 days; Chamber 4, 16 days, and Chambers 1 and 2, 17 days. The amount and severity of infection was estimated by the standard method and the results are summarised in Table VII.

As was to be expected, the greatest amount of infection occurred in Chamber 6 at a constant temperature of 34–35° C., and these plants also showed the greatest number of heavily infected leaves, *i.e.* Classes I and II. The plants sprayed at a lower and subsequently exposed to a higher temperature, Chambers 3 and 4, showed considerable reduction in the severity of infection, but the degree of infection as judged by the more severe classes still remained fairly high. The difference between these two chambers is not significant and one must conclude that an additional 24 hours at a low temperature after spraying had no appreciable effect on the ultimate infection. The plants sprayed at a high temperature which was then reduced, *i.e.* Chambers 1 and 2, showed a great decrease in the amount and severity of infection. Here again there is no significant difference between the two sets of plants. The plants in Chamber 5 which

were exposed to a regular diurnal variation of temperature showed an amount of infection less than those in Chambers 3 and 4, but considerably more than those in Chambers 1 and 2; that is to say, a degree of infection corresponding approximately to the amount to be expected at a constant temperature near to the mean of the variation.

One further experiment was carried out using the alternating temperature thermostat. This experiment also included certain effects of humidity variations which should more properly be considered in the next section, but as the chief results concern temperature changes they may be described here.

Exp. 6. Six-week old plants raised in the glasshouse in the usual manner were employed. These were transferred to the chambers and left for several days at a soil temperature of 30–32° C., and an air temperature of 34–35° C. The chambers were then arranged to give the following air conditions during the experiment:

Chamber 1. Air temperature 34–35° C., constant. Relative humidity 85–90 per cent.

Chamber 2. Air temperature 35° C., constant. Relative humidity 55 per cent. mean.

Chamber 3. As Chamber 2, but plants sprayed once daily with distilled water, commencing 24 hours after spraying. (The humidity was not allowed to rise as a result of this operation.)

Chamber 4. Air temperature 25° C., raised to 35° C. 24 hours after spraying and thereafter maintained constant. Relative humidity 85–90 per cent.

Chamber 5. Air temperature 35° C., but allowed to cool each day for 3 hours during non-illuminated period, beginning 14 hours after spraying. Relative humidity 85–90 per cent., but rose to saturation during each cooling period.

Chamber 6. Air temperature alternating, 23–40° C. Relative humidity 85–90 per cent.

All plants were sprayed with a strong suspension of a young culture of *B. malvacearum* at 6 p.m., that is, one hour after the lights were turned on. Infection developed rapidly, and the plants were examined 9 days later, and the incidence and severity of the infection estimated in the usual manner. The results are summarised in Table VIII.

So far as temperature changes are involved, we are concerned here only with the results from Chambers 1, 4, 5, and 6, in which the humidity was in all cases high, exceeding 85 per cent. Chamber 4, it will be noted,

Table VIII.

Distribution of infection in four classes at the various air temperature and humidity conditions. Exp. 6.

	Chamber 1		Chamber 2		Chamber 3		Chamber 4		Chamber 5		Chamber 6	
	No. of leaves	%	No. of leaves	%	No. of leaves	%	No. of leaves	%	No. of leaves	%	No. of leaves	%
Total no. of leaves ...	159	—	170	—	167	—	170	—	145	—	160	—
Class I, 50 spots and over	6	3.8	0	0.0	1	0.6	6	3.5	11	7.6	9	5.6
II, 25 "	5	3.1	2	1.2	1	0.6	8	4.7	4	2.8	7	4.4
III, 10 "	12	7.6	7	4.1	4	2.4	11	6.5	13	9.0	10	6.2
IV, less than 10 spots	36	22.6	31	18.2	30	18.0	46	27.0	26	17.9	24	15.0
Total no. of leaves infected	59	37.1	40	23.5	36	21.6	71	41.7	54	37.3	50	31.2
Av. no. of spots per leaf	5.66		1.81		1.26		6.21		8.04		6.8	

Table IX.

Distribution of infection in four classes under different conditions of atmospheric humidity. Exp. 7.

	Chamber 1		Chamber 2		Chamber 3		Chamber 4		Chamber 5		Chamber 6	
	No. of leaves	%	No. of leaves	%	No. of leaves	%	No. of leaves	%	No. of leaves	%	No. of leaves	%
Total no. of leaves ...	186	—	159	—	182	—	185	—	174	—	174	—
Class I, 50 spots and over	24	12.9	2	1.3	12	6.6	8	4.3	5	2.9	1	0.6
II, 25 "	5	2.6	4	2.5	6	3.3	10	5.4	6	3.4	3	1.7
III, 10 "	11	5.8	10	6.3	15	8.2	12	6.5	18	10.3	6	3.4
IV, less than 10 spots	25	13.2	24	15.1	35	19.2	22	11.9	26	14.9	28	16.1
Total no. of leaves infected	65	34.5	40	25.2	68	37.3	52	28.1	55	31.5	38	21.8
Av. no. of spots per leaf	11.5		3.07		7.50		6.05		5.25		1.92	

is a duplication of one of the tests carried out in Exp. 5 and it is clear that the results are similar. Comparing the figures for this chamber, where the plants were inoculated at a low temperature which was raised to 35° C. after 24 hours, with those for Chamber 1 (constant temperature of 34–35° C.), it is apparent that there is no significant difference in the amounts of infection; that is, a low temperature at the time of spraying does not influence the final degree of infection if the temperature rises soon after inoculation and remains high thereafter. Similarly, the results for Chamber 5 show that a sudden and brief cooling each day has little effect on infection. The differences between the figures for Chambers 1 and 5 are of doubtful significance, the increase in severe infections in Chamber 5 being offset by the decrease in moderate and very light infections. In the case of the alternating temperature (Chamber 6), the amount and distribution of infection is again not appreciably different from that found at 34–35° C. constant temperature. This is in accordance with the previous results, since in this experiment the maximum of the alternations was 40° C. and the mean approximately 32° C.

THE INFLUENCE OF AIR HUMIDITY VARIATIONS.

It was not found possible to arrange for a satisfactory regular diurnal variation in the humidity of the air within the chambers and the experiments to be described concern only the effect of abrupt changes in relative humidity. Throughout this work the measure used in expressing the moisture condition of the air has been "relative humidity," that is, the "percentage saturation" or the actual pressure of aqueous vapour expressed as a percentage of the saturation vapour pressure at the given temperature. It might be expected on *a priori* grounds that "saturation deficit," *i.e.* the numerical difference between the actual and the saturation vapour pressures at the given temperature, which gives a measure of the evaporating power of the air, would prove to be a better estimate of the air condition for disease studies. Conversion of the figures for relative humidity into saturation deficit in actual experiments in the past shows, however, that the former is more directly correlated with the results of disease estimations than the latter.

Exp. 7. Four to five young plants were raised in each tin, in the glass-house, and the tins transferred to the chambers at a soil temperature of 29° C. and an air temperature of 32° C. Artificial illumination was provided for the usual period of 16 hours daily. These conditions remained constant throughout the experiment. After a few days in the chambers

the plants were sprayed with a strong suspension of the bacteria and thereafter subjected to the following humidity conditions:

Chamber 1. 85-90 per cent. constant.

„	2.	50-51 per cent.	„	
„	3.	85-90 per cent. for 24 hours, thereafter	50-55 per cent.	
„	4.	85-90 per cent. for 48	„	50-55 per cent.
„	5.	50-55 per cent. for 24	„	85-90 per cent.
„	6.	50-55 per cent. for 48	„	85-90 per cent.

The disease developed rapidly, the first symptoms showing in 4 days, and the amount of infection was estimated 9 days after inoculation. The results are summarised in Table IX.

In accordance with earlier results obtained on the effect of constant humidities (5) the plants in Chamber 2 (55 per cent. relative humidity constant) showed a very marked reduction in infection compared with those at the optimum conditions prevailing in Chamber 1 (85 per cent. relative humidity constant). The plants of Chambers 3 and 4 which were inoculated at a high humidity, lowered 24 and 48 hours later respectively, showed approximately the same amounts of infection. This infection, though less than in Chamber 1, was still considerable, showing that the effect of a high humidity at the time of inoculation is not cancelled by a subsequent drop in humidity. Considering now the effect of a low humidity at the time of inoculation it is apparent that provided the humidity becomes high soon after inoculation as in Chamber 5, where the plants were sprayed at 55 per cent. relative humidity raised to 85 per cent. 24 hours later, a fairly high degree of infection can still result. If, however, the humidity remains low for 2 days after inoculation (Chamber 6), then a subsequent rise in humidity does not increase the infection above that occurring in a constantly dry atmosphere.

Exp. 8. Another experiment was carried out as an exact repetition of that just described, except that only five chambers were in use, the test omitted being that corresponding to Chamber 4 in the last experiment; that is, humidity at time of inoculation 85 per cent. dropped to 55 per cent. 48 hours after spraying. Unfortunately, in this experiment, 3 days after inoculation, the main fuse blew on the circuit supplying the whole installation. This accident occurred during the night and as the control of all chambers was thus completely upset for several hours it was decided to remove the plants from the chambers and place them all in the glasshouse. For 3 days, therefore, the plants received the differential treatments and thereafter were all subjected to the same conditions.

The plants were examined for infection 14 days after inoculation and as the results were strikingly in accord with those of the previous experiment it is thought worth while to present a summary of them, in spite of the departure from the usual treatment. For the 3 days of differential treatment the soil temperatures were 29–30° C. and the air temperatures 33° C. in all chambers. The humidity conditions were as follows:

Chamber 1. 85 per cent., constant.

- | | | | |
|---|----|--|---|
| „ | 2. | 55 per cent. | „ |
| „ | 3. | 85 per cent. for 24 hours, 55 per cent. mean thereafter. | |
| „ | 4. | 55 per cent. for 24 hours, 85 per cent. | „ |
| „ | 5. | 55 per cent. for 48 hours, 85 per cent. | „ |

The results obtained are summarised in Table X.

As in the previous experiment, the plants sprayed under conditions of high humidity and subsequently exposed to a drier atmosphere (Chamber 3) still showed a high degree of infection in comparison with those at a constantly low humidity. The plants inoculated at a low humidity but exposed to wetter conditions within 24 hours (Chamber 4) again showed slightly more infection than those of Chamber 2, but this difference is of doubtful significance, except in so far as it runs parallel to that in the previous case. The plants of Chamber 5, which were exposed to the low humidity for 48 hours after spraying, showed the same amount of infection as those at a constant low humidity. The experiment is therefore of interest in adding further evidence in support of the hypothesis that the humidity of the air is only of consequence during the 2 days following inoculation. It is also interesting to note that the general level of infection is distinctly lower than in Exp. 7, which may be explained by the lower mean temperature to which the plants were exposed during the incubation period after removal to the glasshouse.

In the light of these experiments one may consider the results involving humidity differences given in the description of Exp. 6. It will be recalled that in this experiment one chamber (Chamber 2) was run at a low constant humidity, and another (Chamber 3) under similar conditions, except that the plants were sprayed once daily with distilled water without, however, allowing the general air humidity to rise. The results show that the infection in both these chambers was much lower than in any of the other cases, while the spraying did not increase the infection above the control. This is in accordance with the theory put forward in an earlier paper (5) that the importance of humidity lies in its control of the time during which the infection droplets persist. The low

humidity in Chamber 3 caused the fine droplets resulting from the spraying to dry almost immediately, so that no further opportunity for infection to take place was afforded by the treatment.

THE INFLUENCE OF LIGHT AND TIME OF INOCULATION.

It will be remembered that in Exp. 4 a test was made of the influence of time of inoculation with reference to the illumination period on the subsequent infection. The results showed that, with the exception of the plants exposed to an alternating air temperature where the differences were small, the plants inoculated in the dark were, in every case, more heavily infected than those in the light. An experiment with a greater degree of replication was designed to test the truth of this result and also to investigate the general effect of light on infection.

Exp. 9. Plants were raised in the glasshouse in the usual manner in the special tins filled with Gezira soil. The experiment was begun late in the season, and the growth in the glasshouse was slow and somewhat irregular. For this reason the plants were transferred to the chambers for one week at 30° C. air temperature, 30° C. soil temperature, and 17 hours' illumination before inoculation. This resulted in good new growth. The treatments in the six chambers were then as follows:

Chamber 1. Air temperature 35° C. Continuous darkness, extraneous light excluded by sheeting air chamber with brown paper.

Chamber 2. Air temperature 35° C. Continuous illumination.

Chamber 3. Air temperature 35° C. 17 hours' illumination daily. Half total number of plants inoculated in the dark, half in the light.

Chamber 4. As Chamber 3.

Chamber 5. Air temperature 25° C., otherwise as Chamber 3.

Chamber 6. As Chamber 5.

Soil temperatures were maintained at 30° C., and air humidity at 85-90 per cent. relative saturation throughout. The plants receiving no illumination naturally failed to make any further growth, and became very sickly. They were accordingly examined for infection 8 days after inoculation, as any longer period would have resulted in the death of the majority. The remainder of the plants at 35° C. air temperature were examined 11 days, and those at 25° C., 14 days after inoculation. The results are summarised in the usual form in Table XI.

It is clear that plants kept in total darkness were highly resistant to attack, only very few spots being found on the leaves. The theory was suggested in an earlier paper(4) that the degree of attack is correlated

with the carbohydrate content of the leaves, which is in turn conditioned by the temperature and the amount of illumination. In the case of the plants in total darkness, photosynthesis being absent, the available carbohydrates would be rapidly used up and the parasite would be unable to develop in the tissues. The remaining plants at 35° C. constant temperature showed no significant differences in infection whether exposed to continuous light or to 17 hours' illumination daily. Further than this, there was in this case no difference in infection between the plants inoculated in the dark and those in the light. The agreement between the duplicates is unusually close. With regard to the two chambers at 25° C., there is again no definite evidence of a significant difference due to time of inoculation. In Chamber 5 the agreement between the two half sets of plants is fairly close, and though in Chamber 6 there is a rather wide divergence in infection in the two heavier classes, this may have been in part due to the smaller number of leaves in the dark series, affording less opportunity for infection. It would appear, therefore, from this experiment, that the consistent differences in infection with time of inoculation found in Exp. 4 were due to some other factor, at present unknown, than the mere difference in time of spraying with regard to the period of illumination.

DISCUSSION.

The most striking fact which emerges from the experiments described in the present paper is that for two, at least, of the main environmental conditions, namely, soil temperature and air temperature, fluctuations in these factors do not appear to influence the incidence of the disease except in so far as they affect the mean value of the factor. That is to say, fluctuating temperatures, under the conditions prevailing in these experiments, have the same effect on the course of the disease as a constant temperature near to the mean of the variations. This fact is of considerable importance in field studies of the effect of climatic conditions on the epidemiology of the disease, since in all such work it is usually difficult to know whether to take the maximum, minimum or mean as the measure of the temperature conditions prevailing. The demonstration in these experiments that soil type and moisture content of the soil also affect the amount of primary infection under a given set of physical conditions is also of importance in field investigations, and may help to throw light on some of the obscure points in the geographical distribution of the disease.

A further point of note is the elucidation of the effect of the time-factor in variations of the environment. It has been shown by the experiments described that the time-factor in relation to air temperature and to air humidity is entirely different in the two cases. The actual value of the temperature at the time of inoculation is of little consequence, the important consideration being the mean value of the temperature during the incubation period, that is, during the 5 or 10 days (the time depending on the mean temperature) immediately following inoculation. In the case of air humidity the reverse is true, the humidity at the time of inoculation and for a period of 12 to 24 hours afterwards (the critical time again depending on the temperature) being the controlling factor, subsequent variations in humidity having little or no direct effect on the disease. These findings are fully in accordance with the theories put forward in the earlier papers, namely, that the importance of air temperature lies in its differential effect on the balance between the rates of metabolism of the parasite and of the host, while the action of humidity is in controlling the time during which the infection droplets persist.

The influence of soil temperature on primary infection appears to lie more in the direct action of temperature on the parasite, and the present experiments on the effect of abrupt changes in this factor lend further support to this hypothesis. The inhibiting effect of a high soil temperature at the time of sowing is not cancelled by a subsequent drop in temperature, provided the high temperature has prevailed for a sufficient time, about 2 days, to exert its action on the bacteria surrounding the germinating seed. More rapid variations produce only the same effect as a constant temperature near to the mean of the variations, as was pointed out previously.

The experiments described in the present paper conclude the series of investigations which it was proposed to carry out on the influence of environmental conditions on this disease. The method of approach to the problem has been to consider first the effect of a single factor kept at a series of constant values. This has been done for the three main meteorological conditions of soil temperature, air temperature and air humidity, and the results have been described in the earlier papers of the series. The influence of the various factors under constant conditions having been ascertained, it has then been possible to study the effect of changes in the factors during the course of the disease, all conditions save the one picked out for study being still maintained at a constant value, that is, to examine the action of the time-factor in variations of

the environment. Finally, more than one factor has been varied, and the results interpreted in the light of the previous conclusions. With the knowledge so obtained the problem may be transferred to the field, and experiments set up to determine how far the conclusions arrived at can be confirmed under natural conditions. It remains to be seen whether the results of these laboratory trials will be fully borne out by field studies such as those now in progress in Uganda⁽¹⁾.

SUMMARY.

1. Experiments on the influence of variations in the environmental conditions on the bacterial disease of cotton plants caused by *Bacterium malvacearum* are described.

2. A regular diurnal variation in soil temperature is shown to have the same effect on primary infection of seedlings as a constant temperature near the mean of the fluctuations.

3. The mean soil temperature at the time of sowing and for the first few days of germination is the chief controlling factor in primary infection, other factors being equal. Subsequent variations in the soil temperature have little effect on the incidence of the disease.

4. The amount of disease resulting from infection of the seed, *i.e.* primary infection, is higher at soil moisture contents approaching saturation than at normal moisture contents in a given type of soil.

5. The amount of primary infection at a given soil temperature and moisture content varies with the type of soil.

6. A regular diurnal variation of air temperature has the same effect on secondary infection resulting from spray-inoculation of young plants as a constant temperature near to, or slightly above, the mean of the variations.

7. Other things being equal, the amount of infection resulting from spray-inoculation depends upon the mean temperature prevailing during the incubation period of the disease, the actual temperature at the time of inoculation being unimportant.

8. Atmospheric humidity is a conditioning factor in secondary infection only during a short period (less than 48 hours) following inoculation. Its importance lies in its control of the time during which the infection droplets persist. Once penetration of the tissues has been effected variations in the external humidity have little direct effect.

9. Plants kept in total darkness are relatively resistant to infection.

If, as occasionally happens, the disease becomes active before the flowering period of the crop, and the conditions later become dry, many of the affected plants may send up new shoots and these may flower to some extent, but even so the yield is considerably reduced.

If the plants survive long enough to produce pods, the spots develop on them with great vigour and rapidity, and pycnidia of the parasite appear on the lesions in abundance in characteristic concentric zones. The fungus penetrates into the cavity of the attacked pod and reaches the contained seed. Every stage may be found from that in which the seeds are altogether prevented from forming to that in which they mature successfully and are merely marked by a brown patch on the surface. This brown patch may be as large as 1–3 mm., or may be very minute. Sometimes infection is so slight as to be invisible until the seeds are exposed to moist conditions for a day or two.

Severely infected seeds show a low percentage of germination. When the infection is slight to medium, germination is normal.

According to statements of farmers, cattle are unwilling to eat the dried diseased plants. Whether this is due to an undesirable flavour caused by the fungus is not known.

III. FACTORS INFLUENCING DEVELOPMENT OF DISEASE DURING THE GROWING SEASON.

The factors in this connection may be grouped under the two headings of "Environmental" and "Internal." The environmental factors which are of importance are: (a) Rainfall, (b) Temperature, (c) Prevalence of winds, and (d) Cultural practices. The chief internal factor influencing the progress of the disease is the state of maturity of the plant at the time when environmental conditions are favourable. These factors will be dealt with in the order stated.

(1) *Environmental factors.*

(a) *Rainfall.* The rainfall during summer and early phases of growth of the crop indirectly affects the incidence of blight. Thus high rainfall during these periods results in luxuriant vegetative growth of the crop with a thick stand. If later on, viz. during February to April, favourable conditions for the development of the disease prevail, such a crop is more severely attacked than a thin one with less luxuriant growth. This is apparently due to the high humidity in such a crop, and probably also to the greater succulence of the plants themselves.

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The critical period as regards rainfall is however from February to April, which covers the flowering and fruiting periods. The data of rainfall and severity of the disease have been studied in detail for the Attock Tehsil for the years 1920-30. Fig. 1 shows the area sown and the area which failed¹, and Table I gives the rainfall during the growth of the crop for these years.

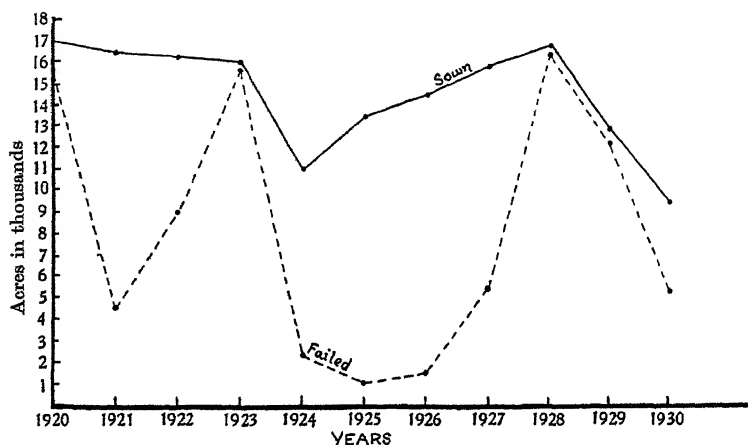


Fig. 1. Area sown and area failed during 1920 to 1930 in Attock Tehsil (Punjab, India).

Table I.

Rainfall (ins.) from October to April for the years 1919-30 at Attock.

Year	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	Total
1919-20	Nil	Nil	5.26	1.78	1.26	2.12	0.66	11.08
1920-21	Nil	Nil	0.72	0.56	1.21	0.15	0.31	2.95
1921-22	0.78	0.09	6.75	0.91	1.21	0.12	1.19	11.05
1922-23	0.10	Nil	1.81	1.31	4.69	4.80	1.86	14.57
1923-24	0.93	Nil	Nil	Nil	5.35	1.56	1.89	9.73
1924-25	Nil	Nil	0.39	1.03	0.68	0.18	0.45	2.73
1925-26	0.75	3.55	Nil	0.40	0.22	6.20	2.62	13.74
1926-27	Nil	0.11	0.09	0.48	1.91	0.64	0.52	3.75
1927-28	Nil	Nil	0.86	1.84	2.05	5.52	1.95	12.22
1928-29	Nil	4.86	2.95	1.91	0.49	0.40	0.65	11.26
1929-30	0.22	Nil	2.29	3.80	0.42	0.12	5.34	12.19
Average rainfall	0.27	0.28	0.72	2.13	2.00	2.27	1.55	9.22

From Fig. 1 it is clear that total destruction of the crop occurred in seasons 1919-20, 1922-3, 1927-8 and 1928-9. Singh(8) mentions that the disease was also very serious in 1925-6, though in fact, as shown in the figure, most of the acreage sown was harvested. A study of Table I

¹ A more satisfactory basis of comparison would be the average yield per acre sown, but such figures are not available.

shows that in all these years in which severe damage occurred, with the exception of the year 1928-9, the amount of rainfall was above the average, and that most of it took place in February and March.

In 1928-9, the weather was unusually cold at the end of January and beginning of February, and during this period frosts of exceptional severity occurred. In the writer's opinion the failure of the gram crop and of many others during that year was primarily due to frost. The blight fungus was prevalent unusually early in the season, viz. in January, and it is reasonable to suppose that it was aided in its parasitism by the weakened state of the plants resulting from the severe weather.

In 1923-4, the rainfall during February was much above the average, but the crop did not fail, or at any rate the greater part of the acreage sown was harvested. There is no record of the prevalence of blight in that year. The discrepancy (if it is real) may be explained as follows. The total failure of the crop in the previous year forced the cultivators to import all their seed from the Mianwali district, where the disease is much less common, and therefore the crop would show much less primary infection.

Table II.

Normal rainfall in different districts of the Punjab from October to April.

District	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	Total
1. (Kangra)	0.79	0.46	1.40	3.74	3.62	3.39	1.93	15.33)
2. (Simla)	0.85	0.58	0.98	2.60	2.90	3.24	2.44	13.59)
3. Rawalpindi	0.53	0.31	0.99	2.36	2.55	2.11	1.66	10.51
4. Gurdaspur	0.43	0.16	0.85	2.30	1.91	1.64	0.81	8.10
5. Attock	0.37	0.23	0.71	1.57	1.49	1.93	1.41	7.71
6. Sialkot	0.33	0.16	0.59	1.71	1.52	1.39	1.0	6.70
7. Gujrat	0.35	0.19	0.56	1.64	1.41	1.51	0.98	6.64
8. Hoshiarpur	0.38	0.17	0.69	1.78	1.57	1.29	0.69	6.57
9. Jhelum	0.31	0.26	0.54	1.49	1.23	1.32	0.97	6.12
10. Ambala	0.67	0.23	0.55	1.62	1.53	0.79	0.46	5.85
11. Jullundur	0.39	0.10	0.55	1.41	1.19	0.97	0.64	5.25
12. Amritsar	0.37	0.10	0.38	1.32	1.17	1.03	0.53	4.90
13. Gujranwala	0.27	0.12	0.50	1.15	0.94	1.08	0.74	4.80
14. Ludhiana	0.44	0.11	0.47	1.27	0.99	0.82	0.61	4.71
15. Karnal	0.40	0.13	0.34	1.04	0.79	0.57	0.41	3.68
16. Lahore	0.25	0.09	0.27	0.91	0.81	0.71	0.43	3.47
17. Mianwali	0.07	0.19	0.22	0.65	0.74	0.83	0.75	3.45
18. Shahpur	0.15	0.16	0.32	0.78	0.89	0.10	0.78	3.18
19. Ferozepur	0.27	0.07	0.23	0.79	0.66	0.66	0.42	3.10
20. Sheikhpura	0.16	0.03	0.30	0.72	0.62	0.71	0.43	2.97
21. Rohtak	0.41	0.07	0.31	0.81	0.47	0.48	0.33	2.88
22. Gurgaon	0.49	0.09	0.25	0.64	0.55	0.41	0.23	2.66
23. Hissar	0.30	0.07	0.28	0.66	0.47	0.46	0.29	2.53
24. Lyallpur	0.16	0.04	0.17	0.43	0.43	0.63	0.51	2.37
25. Jhang	0.10	0.05	0.20	0.44	0.46	0.55	0.54	2.34
26. Montgomery	0.05	0.06	0.21	0.52	0.50	0.43	0.31	2.08
27. D. G. Khan	0.06	0.08	0.15	0.36	0.39	0.41	0.39	1.84
28. Muzaffar Garh	0.06	0.04	0.19	0.34	0.34	0.40	0.35	1.72
29. Multan	0.05	0.06	0.17	0.32	0.29	0.30	0.25	1.44

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The occurrence of blight in the various districts of the Punjab is highly correlated with the amount of rainfall. Table II gives the figures for the months October to April in diminishing order. The horizontal line drawn across the table represents approximately the lower limit at which blight occurs. It should be noted that in the two hilly districts of Kangra and Simla practically no gram is grown. Whether it would be possible to grow it there is doubtful, in view of the heavy rainfall.

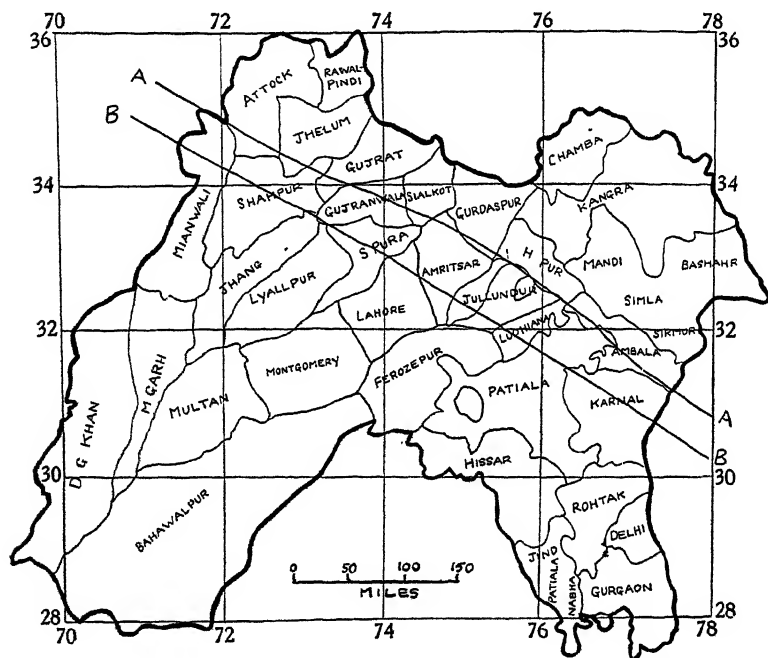


Fig. 2. Showing map of the Punjab with lines A-A (6.0 in. rainfall) and B-B (3.5 in. rainfall) inserted.

In all the others gram is grown extensively but it is only in the districts numbered 3-9 that blight is serious.

Fig. 2 shows a map of the Punjab with the lines of 6 inches (A-A) and 3.5 inches (B-B) rainfall inserted. These lines divide the province into three areas:

- (1) North of the 6 in. line, where blight is serious and in certain parts often epidemic.
- (2) Between the lines A-A and B-B, where blight is reported from time to time, but where it is not serious.

(3) South of the 3.5 in. line, where in some districts it may occur sporadically, but where the damage caused is insignificant.

Fig. 3 gives the average monthly rainfall from October to April (*i.e.* the growing period of the crop) for a number of districts of the Punjab and for the other gram-growing centres of India. The significant point to note is that it is only where the spring rainfall is heavy (*e.g.* Rawalpindi and North-West Frontier Province) that the disease is of importance.

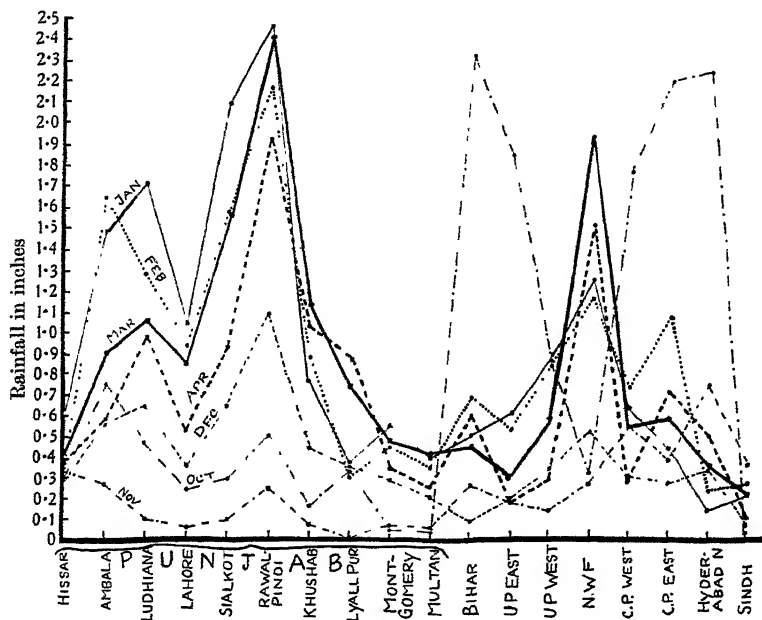


Fig. 3. Average rainfall (October to April) in various gram-growing centres of India.

In such areas as Bihar, Hyderabad and some others, though there is heavy rainfall in October, the disease has not been reported.

(b) *Temperature.* Inasmuch as high temperature is generally associated with low rainfall and *vice versa*, it is obvious that temperature will show some relation to incidence of disease, but the relationship in general is probably only incidental. Nevertheless temperatures above the maximum for growth of the fungus will obviously prevent disease, and such temperatures do occur in certain parts of India, as is shown in Fig. 4. The curves given are those of the average mean shade temperature. The actual temperature round the growing crop is uncertain, but obviously would be higher. The important graphs are those for

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February to April, the months at which flowering and fruiting take place. It is seen that in some parts (Central Provinces and Hyderabad) the mean temperature in March–April comes very close to the maximum temperature (90° F.) for growth of the fungus. Under such conditions infection of the pods and seeds would be impossible, even if a suitable humidity prevailed. In such parts therefore the disease, if accidentally introduced, could not be perpetuated.

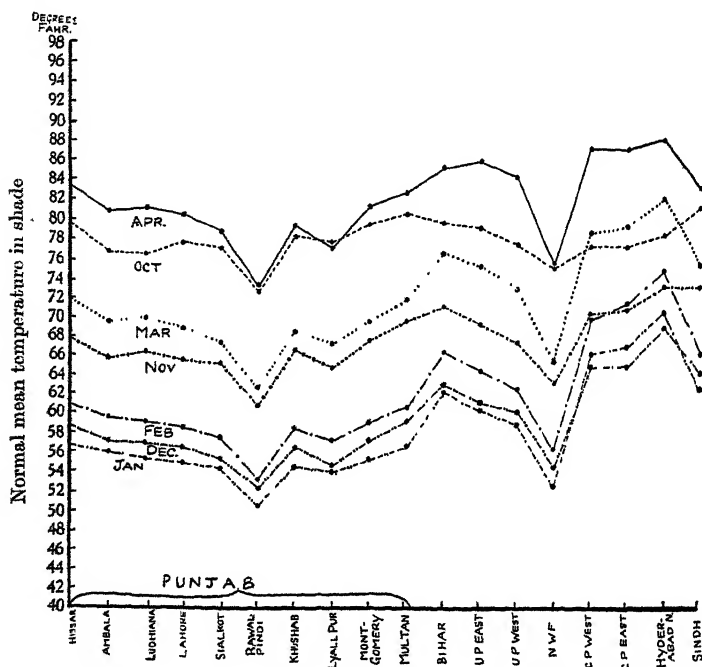


Fig. 4. Normal mean shade temperatures (October to April) in different gram-growing centres of India.

(c) *Prevalence of winds.* Though the fungus of gram blight belongs to a family which does not produce the air-borne type of spore, winds nevertheless play an important part in the spread of the disease. This applies to winds both in dry and wet weather.

With regard to the former, the writer has frequently observed the dried-up leaves and stems of diseased plants being blown for hundreds of yards. These diseased parts bear abundant pycnidia of the fungus, and when they lodge among healthy gram plants, they act as a source of infection whenever wet weather comes on.

In wet weather also wind is a potent agent in disseminating the fungus. The heavy downpours, which are accompanied by thunder and strong winds, cover the fields with a fine mist which is blown to considerable distances. Under such conditions the spores which ooze out from the pycnidia are splashed about and carried away in suspension.

(d) *Cultural practices.* The effect of the system of cultivation explains, in the writer's opinion, the difference shown in the severity of the disease between certain areas which have practically the same climate, e.g. Attock and Rawalpindi. The disease is more serious in the former than in the latter district. The characteristic feature of the system of cultivation in Attock is that gram is the only crop grown on an extensive scale, so that there is practically a continuous stretch of land under this crop. In Rawalpindi, on the other hand, gram is generally sown in mixture with wheat, barley or taramira (*Eruca sativa*), and the fields are separated by fields of other winter crops. It is clear therefore that the conditions are less favourable for the spread of the disease in the latter case.

(2) *Internal factor.*

As has been already stated, the most critical period for the gram crop in relation to blight is February to April when flowering and fruiting take place. Canizo⁽⁴⁾ in Spain also mentions that the blight usually appears at the flowering stage of the plant. This is not wholly due to weather conditions, but partly to the fact that the plant becomes more susceptible as time goes on. Thus experiments have shown that young seedlings are more difficult to infect than older ones. The following is the record of such an experiment:

Batches of seed were sown at intervals and grown in a greenhouse at 55–80° F. temperature. On May 2nd, 1932, when the plants were sprayed with a suspension of spores of *Ascochyta Rabiei*, the various batches were 10, 8, 5½ and 3 weeks old. The sprayed plants were kept moist under bell-jars for 4 days. After 15 days the following results were shown:

The leaves of the youngest plants were only slightly attacked, and only 1 per cent. of the total number of leaves on the plants were shed. Slight to medium infection of the young stem tips occurred. The plants of the other batches were severely attacked and showed 100 per cent. infection on the leaves, 75 per cent. of which were shed. Infection of the stems was also severe.

Various other inoculation experiments also showed that gram plants when inoculated at the flowering stage were completely scorched, whereas

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young seedlings showed very slight infection. In experiments carried out from December to February with plants grown under glass at about 20° C., it was found that even plants 5-6 weeks old were difficult to infect. Thus from all these experiments it is evident that the plant shows increased susceptibility with age.

A well-known feature of the gram plant is its capacity to give out an acid secretion from glandular hairs on its surface. Sahasrabuddhe⁽⁷⁾ has shown that this secretion consists almost entirely of malic acid (with traces of oxalic and acetic acids) and that it increases with age of the plant, reaching its maximum at the time of flowering and fruiting. His work deals with conditions in Bombay, which are different from those of the Punjab and North-West Frontier Province where blight is serious. Nevertheless the experience of the cultivators and of the writer is in agreement with Sahasrabuddhe's results. If one walks barelegged through a crop at about the flowering and fruiting time, one experiences a stinging feeling which is absent when the crop is younger. The taste of the leaves likewise becomes sharper as the plants become older, and it is for this reason that from about the middle of February the peasants cease to use leaves of the plants as a vegetable.

This acidity was roughly determined on plants used in the experiment quoted on p. 621. Control plants, at the time of inoculation, were sprayed with distilled water and kept (covered) under bell-jars for 6 hours, after which the drops of water from leaves of the plants were removed by means of pipettes, and the pH and normality of the acid liquid thus collected estimated. The results obtained are given in Table III.

Table III.

The pH and strength of the acid liquid on plants of different ages¹.

Age of the plant in weeks	pH	Strength
3	4.2	N/210
5½	3.6	N/100
8	3.5	N/76
10	2.8	N/52

From these results it is clear that drops of distilled water left on leaves of gram plants become highly acidic, and that the available acid increases with the age of the plant.

During these and various other tests there were indications that the conditions (temperature and light) under which the gram plant is grown

¹ The results should be taken as comparative ones only as the degree of acidity will depend upon the size of the drop as well as the time for which it is allowed to remain on the plant.

affect the secretion of the acid by the plant. Till all such factors are worked out the results obtained in one country cannot be definitely applied to another.

The significance of these acidic relationships is that the gram blight fungus is much favoured in its germination by the presence of acid. The writer has shown elsewhere (v. p. 614, footnote 1) that germination is very slow and uncertain when the spores are transferred directly to drops of distilled water.

It was thought first of all that this behaviour was similar to that described by Butler(3), who states that spores of *Colletotrichum Lindemuthianum* Br. and Cav. do not germinate freely unless the mucilaginous mass in which they are embedded is removed by washing. To test this point, spores of *A. Rabiei* were shaken in water and in various concentrations ($N/200-N/10$) of sulphuric, hydrochloric and malic acids. The suspensions were then poured on to filter papers which held the spores back. The latter were washed several times with distilled water and finally placed in drops of pure water on slides. None of the suspensions so prepared germinated freely.

The effect of acidity on germination was now tested. Solutions of strengths $N/50$, $N/25$ and $N/10$ of the following acids were prepared—oxalic, malic, tartaric, citric, hydrochloric, nitric and sulphuric—and spores added to each after a preliminary shaking in water. Malic and tartaric acids were the only ones in which the spores germinated.

Further experiments showed that for malic acid the optimum concentration was in the neighbourhood of $N/50-N/25$. The data obtained on this point are given in Table IV.

Table IV.

Germination of spores of A. Rabiei in different concentrations of malic acid.

		Strength of acid					
After 48 hours:	Water	<i>N</i> /50	<i>N</i> /25	<i>N</i> /12	<i>N</i> /7	<i>N</i> /3	<i>N</i> /1.4
% germination ...	15	95	95	25	10	Nil	Nil
Average G.T. length (μ)	3.1	162.8	205.1	32.2	2.4	Nil	Nil

The pH of solutions of $N/50$ to $N/25$ ranges from 2.6 to 2.4.

When such acids as oxalic, citric, hydrochloric, nitric and sulphuric were used in concentrations giving a pH of 2.5, they were still unable to cause germination. When however glucose was added to acids of such concentration, good germination resulted. Table V illustrates this

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point, as well as the fact that such nutrients as potassium chloride potassium nitrate and potassium phosphate (K_3PO_4) are of no value in this connection.

Table V.

Germination of spores of A. Rabiei in various media.

Medium	pH	% germination after 96 hours
2 % KCl	7.0	2
2 % K_3PO_4	9.0	2
2 % glucose	6.8	10
HNO ₃ alone	2.5	Nil
2 % KCl + HNO ₃	2.5	Nil
2 % K_3PO_4 + HNO ₃	2.5	Nil
2 % glucose + HNO ₃	2.5	100
HCl alone	2.5	Nil
2 % KCl + HCl	2.5	2
2 % K_3PO_4 + HCl	2.5	Nil
2 % glucose + HCl	2.5	100
H ₂ SO ₄ alone	2.5	Nil
2 % KCl + H ₂ SO ₄	2.5	Nil
2 % K_3PO_4 + H ₂ SO ₄	2.5	Nil
2 % glucose + H ₂ SO ₄	2.5	100
Oxalic acid alone	2.5	Nil
2 % KCl + oxalic acid	2.5	Nil
2 % K_3PO_4 + oxalic acid	2.5	Nil
2 % glucose + oxalic acid	2.5	100

The optimum pH for germination in glucose + hydrochloric acid was found to be 2.4, *i.e.* the same as for malic acid. The results illustrative of this point are given in Table VI.

Table VI.

*Germination of spores of A. Rabiei in 2 per cent.
glucose solution of different pH.*

pH	After 48 hours	
	% germination	Average G.T. length (μ)
2.1	Nil	Nil
2.4	95	38.4
2.8	70	9.8
3.9	70	12.4
4.4	60	8.4
5.1	25	3.5
6.8	20	4.1
7.8	20	5.2
9.2	10	3.6

It is clear from this that germination of the spores of *A. Rabiei* is favoured by a rather high acidity together with the presence of a carbon food. A high concentration of malic acid presumably acts in both capacities.

The optimum temperature for germination of spores as tested in *N/25* malic acid lies between 15° and 25° C., the curve being very flat over all this range. Germination is also good at 30° C., but does not take place at 33° C. The lower temperature limit for germination lies below 10° C., but it was not definitely determined.

IV. TRANSMISSION OF DISEASE FROM ONE YEAR TO ANOTHER.

Observations show that there are at least four possible modes of transmission of the disease from one year's crop to the next, viz.:

- (1) By infected seed.
- (2) By seed contaminated with spores on the surface.
- (3) By seed mixed with plant parts bearing pycnidia of the fungus.
- (4) By soil infection.

These various methods will now be discussed in the order given.

(1) *Seed infection.*

In any sample of seed taken from a diseased crop every gradation is seen from apparently healthy and fully developed seeds to those which are shrunken, discoloured and with large lesions on their surfaces. From such discoloured seeds, whether slightly or severely affected, the fungus has been repeatedly isolated by the usual methods.

When severely infected seeds are sown, germination as a rule does not take place. If the seeds show only a small discoloured spot, they germinate but a considerable number of the seedlings (in various experiments about 60 per cent.) are diseased. The precise symptoms shown vary somewhat according to the conditions. Thus the young seedlings on appearing above ground may show infection of the tip or any part of the young stem. In other cases no infection may be visible until the seedlings are about two months old. The conditions underlying this variability have not been worked out.

The appearance of lesions some considerable time after the seedlings have appeared above ground is somewhat suggestive of a smut-like method of attack. Sections of seedling stems which have behaved in this way have been examined both microscopically and by the method of plating, but no fungus has been demonstrated.

No pycnidia have been seen on infected seeds at the time of sowing. When, however, such seeds are dug up about a week after sowing they almost invariably show mature pycnidia with spores oozing out.

(2) *Contamination of surface of seeds with spores.*

There is abundant opportunity for this to take place in the process of threshing. It is not practicable to rogue out the diseased plants in the crop and thus healthy and infected plants are mixed together during harvesting. Threshing is done by the treading of bullocks on a floor exposed to the open air. In this operation the crop is liable to be wetted by dew or rain, with the result that spores are liberated from the pycnidia and come in contact with healthy seeds. It is not improbable that the mucilaginous material in which the spores are embedded serves the purpose of fixing the spores to the seed coats.

The question now arises as to whether such spores are able to live during the summer months (June to September) which elapse before the next seeding time. The following experiment was set up to test this point:

Seeds of gram were smeared with spores of *A. Rabiei* and then incubated in batches at 25°, 30° and 35° C. for five months. They were then shaken in a N/25 solution of malic acid to liberate the spores and the germination of the latter tested. Germination was found to be 50 per cent. for the spores exposed to 25° and 30° C., and 5 per cent. for those at the higher temperature. As the conditions of summer storage in the Punjab are such that temperatures of 35° C. are only reached occasionally and for short periods, it is clear that a fairly large percentage of the dried spores contaminating the surface of seeds will remain viable till seeding time.

Numerous experiments have shown that seeds smeared with spores and then sown give rise to infected seedlings in a high percentage (60 to 100 per cent.) of cases. The symptoms shown are similar to those obtained with the naturally infected seed.

(3) *Contamination of seed samples with plant parts bearing pycnidia of the fungus.*

Seed samples obtained by the writer from districts where the disease is prevalent have been found invariably to contain broken pieces of plant tissue bearing mature pycnidia. From such material cultures of the fungus have been prepared. The method of winnowing adopted is crude, and it is in fact difficult to separate short pieces of stems from the seed. Such infected tissue is obviously a potential source of contamination in the seed bed.

(4) *Soil infection.*

This possibility has to be considered, especially in cases where gram crops are grown year after year on the same land. The readiness with which infected dried-up parts of plants are blown on to otherwise clean land has also to be borne in mind in this connection. So far the writer's attempts to produce soil infection have given little success, but inasmuch as secondary infection under suitable conditions is so potent, even an occasional carry-over by this means is not to be ignored.

It is impossible at present to estimate definitely the relative importance of these four methods of perennation, but in the writer's view the first two are the most important. Again, actual seed infection is probably less important than seed contamination, as the latter will take place in crops which are not necessarily severely infected. In estimating the possibility of soil infection one has to bear in mind the lack of humus in many gram soils and the high summer soil temperatures (30° to 40° C. for three months), both of which factors are unfavourable for the growth of the fungus as a saprophyte. Whether the fungus can remain viable in a dormant condition in diseased tissues through the summer period is not known¹.

V. CONTROL MEASURES.

These will be discussed under the following headings:

- | | |
|--------------------------|--------------------------|
| (1) Use of healthy seed. | (4) Resistant varieties. |
| (2) Seed treatment. | (5) Cultural practice. |
| (3) Spraying. | |

(1) *Use of healthy seed.*

Since blight would appear to be perpetuated mainly through infected or contaminated seed, an obvious control measure is the use of clean seed. As has been shown in Fig. 2 (p. 618), the seed produced in localities south of the line *B-B* is almost free from the disease. The districts of Hissar and Ferozepur, which enjoy a climate very unfavourable for blight and which are well removed from any blight-infested area, would prove very suitable for this purpose. If however the cost of transport should prove to be a serious objection to this plan, it might be feasible to establish

¹ Since preparation of the text, the writer has found (September, 1932) in the Campbellpur district of the Punjab debris of gram plants from which in many cases the typical gram blight fungus could be isolated. This material was derived from a crop harvested in the preceding April, whence it is clear that the fungus can remain viable in this way over the summer period.

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a seed farm in a suitable locality of the district of Mianwali which offers the advantages of nearness to the severely affected areas and of virtual absence of disease.

Such schemes would necessarily involve certification of seed samples for freedom from the parasite. The elaboration of a satisfactory laboratory culture test would offer no difficulties.

The use of clean seed on a small scale has been tried by Singh (8, 9, 10) and by Mohendra and the writer on a number of occasions, but with little useful result. The crop from such seed was definitely later in showing blight than the surrounding crops, but it succumbed in the long run. The reason for this is not difficult to understand when the ready means of secondary infection are borne in mind. The use of clean seed, if it is to be effective, must be general over the whole of the infected areas, and this will obviously require a measure of official compulsion. Considering the heavy losses entailed in the form of remission of land revenue, the Government should have every inducement to attempt such a programme.

(2) *Treatment of seed.*

The scheme advocated under (1) would involve extra expense to the cultivators, and furthermore is not yet in being. Such being the case, attempts should be made to treat seed from infected crops so as to eliminate as far as possible sources of primary infection. The writer has carried out experiments devised to test the usefulness of the following methods:

- (a) Steeping seed in copper sulphate solution.
- (b) Dry heat treatment.
- (c) Hot water treatment.

(a) *Copper sulphate steep.* This treatment would only control infection as brought about by spores adhering to the seeds.

Spores of *A. Rabiei* were kept for 10 minutes in 5, 2, 1, 0.5, 0.25 and 0.1 per cent. copper sulphate solutions. They were then cleared from the toxic solutions by filtering and washing and their germination in dilute malic acid solution tested. It was found that a concentration of 0.25 per cent. killed all but about 1 per cent. of the spores, while the higher concentrations were completely effective. Germination of the seed was not injured by any of the concentrations used¹. It is suggested

¹ Canizo (4) in Spain suggests the use of a 0.5 per cent. solution of copper sulphate. He states that higher concentrations cause severe injury to the seed. Perhaps there are marked differences from one variety to another in this respect.

therefore that steeping of seed in a 0.5 per cent. solution of copper sulphate for 10 minutes should be tested on an extensive scale under local conditions.

(b) *Dry heat treatment.* In North-Western India during summer the temperature of gram seeds can be raised to as high as 60° C. if the latter are mixed with dry sand or ashes and exposed to the sun. To test whether such a treatment would kill the parasite (hibernating mycelium) if present, seeds showing discolorations were heated to 60° and 68° C. for various periods varying from 6 to 24 hours. The seeds so treated were not injured in any way, but it was found that even after the longest treatment given the fungus was viable, as tested by culturing on oatmeal agar.

(c) *Hot water treatment.* Seeds showing the characteristic discolorations due to the fungus were soaked in water at 20° C. for 6 hours, and then in hot water at 40°, 45°, 50°, 53° and 55° C. for 10 and 15 minutes. Parallel batches of healthy seeds were similarly tested. The results showed that the fungus was not killed at any of the temperatures below 53° C. At 53° C. the fungus was killed in 70 and 95 per cent. of the cases when the duration of treatment was 10 and 15 minutes respectively. Germination of the seeds, healthy or infected, was not reduced. At 55° C., though the fungus was killed in all cases, the percentage of germination was only about 20.

The efficacy of the hot water treatment would obviously depend on the depth of the fungal lesions occurring on the surface of the seed, and as a practical measure it would seem probable that it would be best applied to seed which is not severely infected. It might thus be applied on seed farms to clean up a stock of slightly infected but otherwise valuable seed.

In connection with seed treatments, the writer has tried the effect of dusting seed with malic acid with the idea that the fungus if present would show increased parasitism and thereby prevent infected seedlings from coming above ground. The treatments given and the results shown were as follows:

- (i) Seeds dusted with malic acid—Normal germination and growth.
- (ii) Seeds smeared with spores of *A. Rabiei*—Normal germination and growth, but 80 per cent. of the seedlings showed lesions of the disease on stems.
- (iii) Seeds as in (ii) but dusted with malic acid—Germination normal, but all of the seedlings, when 1-1½ in. high, shrivelled up as a result of severe infection.

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Whether in practice the depth of sowing could be arranged so as to ensure that acid-treated seeds carrying the fungus would fail to appear above ground and, more generally, whether there is any practicability in the method remains to be seen. At any rate the results obtained in this connection afford an interesting confirmation of the intensification by malic acid of the parasitic vigour of the fungus.

(3) *Spraying.*

Canizo(4) in Spain recommends the spraying of the crop with 2 per cent. Bordeaux Mixture, one application to be given before and the other after flowering. Under Indian conditions this method is impracticable on account of the expense involved.

A test was made by the writer in the laboratory in which gram plants (9 weeks old) were sprayed with a 2 per cent. lime solution in order to neutralise malic acid on the plants, and then inoculated with a spore suspension of *A. Rabiei*. Though the infection on plants sprayed was very slight, the leaves were somewhat scorched by the lime. Whether it would be possible to find a suitable strength of any alkali which would be effective in controlling the disease without injuring the plants cannot be said at present.

A further practical difficulty in using spray solutions, even if a very cheap one were available, is the scarcity of water in many of the gram-growing areas. As has been already stated, the soils are extremely sandy and the only sources of water, even during the rainy period from February to April, are deep wells, which in many cases are at a considerable distance from the fields. It would therefore seem that there is more promise to be had in methods of dusting.

(4) *Resistant varieties.*

Singh(9, 10) tested the resistance of nearly all the available types of gram by growing them in the neighbourhood of infected crops. He found that some varieties showed relatively little disease in the first year of trial, but that the intensity of disease increased in subsequent years. He suggested the possibility of a new and more parasitic strain of the fungus arising. In the writer's view there is no need to make this hypothesis, as the effect can be fully explained by the natural spread of the disease in course of time.

He found that Punjab gram type No. 7, though not sufficiently resistant as to save the situation in the affected locality, was somewhat more resistant than all the other types. The writer has tested this type

by inoculating the plants at flowering stage with spores in suspension of *A. Rabiei* and has always found it to be very susceptible. In fact in all the experiments described in this paper this was the only type used.

Labrousse (8), working at Versailles (France), states that 11 out of 167 types of Chick-pea showed definite resistance to the gram blight disease, but none of the Indian types tested by him was resistant.

In testing the resistance of gram types to blight, inoculations should be made at the flowering and fruiting stage, otherwise even plants of a susceptible variety may show a deceptive appearance of resistance.

(5) *Cultural practice.*

In view of the evidence now available of the capacity of the fungus to remain viable over the summer in the debris of the preceding season's crop (p. 627, footnote), it is advisable that a system of rotation should be followed wherever possible, and that efforts should be made to dispose of all refuse of the preceding crop lying on the ground.

VI. SUMMARY.

1. The importance and distribution of blight of gram are described.
2. The field appearance of blight, and the influence of environmental factors such as rainfall, temperature, wind and cultural practices on its incidence and development have been studied. The occurrence of blight in various parts of India is shown to be highly correlated with the amount of rainfall during the flowering and fruiting periods of the gram crop.
3. It is shown that the gram plant increases in susceptibility with age and is most susceptible at the flowering and fruiting stages. This susceptibility has been found to be directly proportional to the amount of malic acid secreted by the plant on its surface.
4. Germination of spores of *A. Rabiei* has been studied in some detail, and it has been found that they are favoured in their germination by the presence of N/50-N/25 malic acid or acidified carbon food (pH 2.5).
5. Modes of transmission of blight from one year to another are described.
6. Measures to control blight are given and some new lines of attack have been indicated.

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STUDIES ON *PENICILLIUM CHRYSOGENUM* THOM, IN RELATION TO TEMPERATURE AND RELATIVE HUMIDITY OF THE AIR

By PERCY GROOM, F.R.S.

AND

THÉRÈSE PANISSET, B.Sc.

(*Botany Department, Imperial College of Science
and Technology, London.*)

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INTRODUCTION.

It is well known that the covers of books undergo disintegration when exposed to light and excessively dry air, or when mildew develops on them in the presence of moisture. This investigation, originally begun at the request of the authorities of the Public Record Office and subsidised by the Department of Scientific and Industrial Research, had the practical aim of determining the minimum hygrometric conditions requisite for the mildewing of book materials.

The materials used in this research came from the Public Record Office, and the mildew present in that building was in most cases caused by *Penicillium chrysogenum*, determined in accordance with C. Thom's *The Penicillia* (3). This species alone was investigated therefore, and, excepting where specially recorded, all the statements in this paper refer to it: but as will be seen later the co-operation of other fungi and bacteria in the mildewing was not excluded.

Part I of this paper deals with the first essential for mildewing, the germination and viability of the conidia: Part II treats of the development of macroscopic mildew.

PART I. GERMINATION AND VIABILITY OF CONIDIA.

During the progress of the present research, Tomkins(4), investigating the interaction of the two variables, relative humidity and temperature of the atmosphere, determined with precision the minimal values of these at which germination of the spores of various fungal species can take place. References to his results are made later in this paper, but here it may be noted that in his work with spores of *Alternaria citri* the lowest relative humidities at which germination took place were 89.3 and 83.8 per cent. on glass and on Dox's dried agar respectively, at 30° C.

(a) *Germination in water, saturated air and malt agar.*

The first experiments were designed to obtain information as to the power of conidia to germinate in distilled water and saturated air, and as to the effect of temperature and food material on such germination. Conidia of *Penicillium chrysogenum*, and also of *Aspergillus niger*, were germinated at constant temperatures: (1) in distilled water in hanging-drop cultures; (2) in saturated air in sealed van Tieghem cells; (3) on films of 2 per cent. malt agar. Table I records the number of hours that had elapsed before germination was first seen.

Table I.

Temp. ° C.	Hours required for germination					
	In water				On malt agar	
	P.		A.		P.	A.
	Sat.	W.	Sat.	W.		
10	69	53	—	—	41	—
15	42	44	288	69	24	44
20	22	22	55	23	15.5	15.5
25	16.5	16.5	23	16.5	12	9
30	23	23	17.5	16.5	17.5	6
35	—	83	53	22	42	6
40	—	—	—	25	—	7
45	—	—	—	—	—	16.5

P. denotes *Penicillium*; A., *Aspergillus*; Sat. = saturated air; W. = distilled water.
Short dashes (—) denote that no germination took place.

These results as regards *P. chrysogenum* proved that the conidia germinate in distilled water and in saturated air, and that in both these media the latent period at the same temperature was the same, excepting at the extreme temperatures (10 and 35° C.) where there was a lag in the saturated air. In each medium the optimum temperature for inception of germination was nearest to 25° C. and the maximum between 35 and 40° C., except in saturated air where it sank below 35° C. Above and below 25° C. the latent period of germination increased with increasing

distance from this temperature. On agar the same general results were obtained, but at the same temperatures the latent periods of germination were shorter. *Aspergillus* sp. (*A. niger*?) yielded similar results, but the three cardinal temperatures were higher, and the lag in saturated air was more considerable excepting near the optimum.

(b) *Germination in moist air on glass.*

In all cases the conidia were placed on glass coverslips in sealed glass vessels; the temperatures maintained were mostly constant and ranged from 1 to 40° C., but in a few cases they were the varying temperatures of the laboratory (15–25° C. or 20–25° C.); the relative humidities ranged from 100 to 54 per cent. The methods by which these were secured is given below¹. The conidia used were always 1–3 days old excepting when the temperatures were 10 or 15° C., in which cases they may have been sometimes older.

The results are recorded in Table II, in which × and 0 respectively denote the occurrence or non-occurrence of germination.

Table II.
Range of germination with temperature and humidity.

Temp. ° C.	Relative humidity (%)					
	100	95–90	91	87–83	81	Less than 80 (78–0)
40	0	0	0	0	0	0
35	×	×	×	0	0	0
30	×	×	×	0	0	0
25	×	×	×	×	×	0
20	×	×	×	×	0	0
15	×	×	×	×	0	0
10	×	×	×	×	.	.
8	×	.	×	0	0	0
1	×	.	×	0	0	0

¹ *Solutions used to induce relative humidity of the atmosphere.*

A. *Sulphuric acid* (see Landolt and Bornstein⁽¹⁾):

Rel. hum. (%)	H ₂ SO ₄ (% by volume)	Rel. hum. (%)	H ₂ SO ₄ (% by volume)
96	5	68	22.5
91	10	61	25
83.5	15	54	27.5
81	16	26	40
78	17.5	20	43
72.6	20		

B. *Saturated salt solutions* (see Spencer⁽²⁾):

Salt	Rel. hum. (%)		
	10–15° C.	20–25° C.	30–35° C.
KNO ₃	95	94	93
KCl	87	86	85
NaCl	77	76	75

The minimum relative humidity of the air in which germination took place was 81 per cent. at 25° C., but increased proportionately as the temperature diverged from this in an upward direction to 35° C. or downward to 1° C. Thus the *optimum* temperature was found to lie near 25° C. As regards the *maximum* temperature for germination in moist air these results agree with those previously described in indicating that this lies between 35 and 40° C. The *minimum* evidently lies below 1° C. (0° C. was not tested).

The results obtained in these experiments as regards the *latent period of germination* in different atmospheres confirmed and amplified those obtained with conidia germinated in van Tieghem cells.

Table III summarises these results by giving the minimum *observed* number of hours that had elapsed before germination had taken place in the various atmospheres. The number of hours do not represent true minima, as it was impossible to examine the conidia at repeated short intervals during day and night and at the same time to keep the atmospheres at constant temperatures and relative humidities.

Table III.
Hours required for germination.

Temp. ° C.	Relative humidity (%)											
	100		95-90		91		87-83				83-5.	
10	96	48	120	.	192	192	1056	.
15	72	72	96	72	120	168	120
20	47	96	.	336
25	.	30	48	.	96	.	216	552	.	408	.	576
30	.	24	48	189
35	.	.	72
15-25	216
20-25	.	.	72

In Table III the figures given in each narrow vertical column represent one and the same experiment and indicate that at the same constant relative humidity as the temperature approached 25° C. the latent period shortened. Each horizontal series of figures represents a number of different experiments (resulting from different batches of conidia), so that the results are obviously disturbed by irregularities due to extraneous factors, including probably the properties of the conidia. Nevertheless they show that, in general, at the same constant temperature as the relative humidity declined the latent period of germination lengthened.

The longer latent periods of germination, compared with those obtained in the same atmosphere (saturated air) and with the use of

van Tieghem cells, were probably due in part to the longer time required for the air to acquire its full humidity in the larger glass vessels, but conceivably also to the occasional opening of these vessels for examination of the conidia. A few simple experiments showed that, within the limits of the method, the opening of the vessels caused no significant change in the lengths of the latent periods. But under the same atmospheric conditions, differences in the latent period of germination strongly suggested that the individual conidia differed in their properties.

The first obvious possible cause of these differences is *age* of the conidia. In experiments conducted in atmospheres of relative humidity 91 per cent., the conidia whose behaviour is recorded respectively in the left-hand and middle columns of Table III under the heading of 91 per cent. were respectively from old and young cultures, yet there was no corresponding difference in the latent periods. But here it is conceivable that in both cases only new young spores germinated.

To demonstrate this observations (by Dr Phyllis Hicks) were made on the percentage of conidia from old and young cultures that germinated in different media at the same temperature. In the first place, from a culture of *P. chrysogenum* 67 days old on malt agar, conidia were sown as follows: 121 in distilled water, 43 in saturated air, and 70 and 50 on malt agar. The cultures kept at a constant temperature of 25° C. yielded the results shown in Table IV. Incidentally the results showed the shortening of the latent period by the supply of nutrient agar in place of water.

Table IV.

Percentage germination of 67 days old conidia.

Hours after sowing	Percentage of spores germinating (approx.)			
	In water	In sat. air	On agar	
18	1.6	2.3	90	90
22	1.6	2.3		
24	2.5	2.3	95.5	98
42½	2.5	9.3		
46½	2.5	9.3	100	100
67	8.3	9.3		
71	9.2	9.3		
91	13.3	Discontinued		
161	22.5			
190	33.3			

In the second place conidia of the same old culture were sown on malt agar, and from the resultant mycelium the earliest conidia produced in the first night, and therefore aged less than 24 hours, were germinated

in water at 25° C. After 23 hours in water the percentage germination was 40, after 27 hours 70, after 29 hours 94·3 and after 50 hours 98·4. These results appeared to indicate that with increasing age of the conidia there is a lengthening of the latent period of germination when the spores germinate in water, but that this effect of age is concealed when the conidia germinate on nutrient agar.

Despite the obvious limitation of the method, in view of the difference in the ages of the conidia on the old culture, new trials were made by germinating conidia of cultures 3 days, 120 days and 210 days old, in water and on 2 per cent. malt agar at 20° C.; in water three different sets of spores from 3 days old cultures were tested. After 24 hours, the percentage germination on 2 per cent. malt agar was 100 whether the spores came from 3, 120 or 210 days old cultures, while in water spores from 3 days old cultures gave 0·6, 12 and 29 per cent. germination and from the 210 days old cultures 1·6 per cent. germination. There was thus no proportion between the percentage of germination, either in water or on agar, in 24 hours and the age of the culture.

The fate of the conidia in water was traced further by continuing observations for additional periods in hours as indicated in Table V.

Table V.
Percentage of germination.

Age, in days, of culture	Hours in water								
	24	48	72	96	120	144	168	192	264
3	29	45	45	68	—	—	T.L.	—	—
3	0·6	14	14	20	—	—	T.L.	—	—
3	12	25	—	—	—	—	—	—	—
210	1·6	—	—	4	6·5	13	16	21	24

T.L. indicates that the hyphae had grown too long to allow accurate counting of the conidia.

Although these results harmonise with the conception that age lengthens the latent period of germination in water, the very unequal results obtained from the three different cultures 3 days old suggest the action of some additional factor. Whatever be the intervening cause it is clear that conidia produced on 3 days old cultures grown in similar general external conditions cannot be assumed to be exactly alike in properties; and this conclusion is confirmed in the investigation, described later, concerning the effects of exposure of the conidia to different atmospheres. These experiments thus do not prove that age (and exposure to air) lengthens the latent period, but they do show that the conidia are liable to vary widely in their capacity for germination.

(c) *Effects of time of exposure to different atmospheric conditions before germination on the germinative characters of conidia.*

In view of the ambiguity involved in the method just described, a number of sets of experiments were made with conidia obtained solely from cultures 3 days old grown at 20° C. on 2 per cent. malt agar. But even here a difficulty presented itself as the conidiophores and conidia on the cultures were largely in contact with drops of an aqueous solution excreted by the fungus. In order to remove the excreted water the cultures were exposed for 1-2 hours to calcium chloride so that the water evaporated before the conidia were collected. It was found that after this treatment 100 per cent. of the conidia germinated on 2 per cent. malt agar at 20° C., but there is no certainty that the drying treatment had not affected some of the conidia to such an extent as to change the length of the latent period *in water* (see later).

The conidia were then collected and exposed for various periods to atmospheres of various constant relative humidities and a temperature of either 20 or 30° C. The lengths of exposure ranged from 1 to 136 days, and the relative humidities from complete dryness (for the sake of brevity recorded below in the tables as 0 per cent., and induced by the use of pure sulphuric acid or phosphorus pentoxide), to 26 and 72.6 per cent. Thereafter they were placed to germinate at 20° C. on 2 per cent. malt agar or in distilled water, and the percentage germinations were determined after 24 hours on agar and in water, and after longer periods in the latter.

The results are set forth in Table VI, which records in the left-hand column the number of days of exposure to the atmosphere to which the conidia were exposed, and in all the other columns the percentage germination in 2 per cent. malt agar (column 2) or in distilled water (the remaining columns) after the lapse of the number of hours indicated at the heads of these columns.

For the estimation of percentage germination during periods exceeding 24 hours, distilled water was used as the medium because on agar the rapid growth of sporelings soon rendered it impossible to observe the fate of the other spores. The number of spores counted to determine the percentage germination at any one time ranged from 2000 down to 66, but for the most part ranged between 1000 and 150.

In the table the letters T.L. indicate that in water sporelings had produced hyphae so long as to prevent accurate counting of germinated and ungerminated conidia. These sporelings prove that some of the

conidia allowed the escape of soluble nutrients into the water, which thus by no means continued to be distilled water.

Percentage of germination in 24 hours.

With increased length of exposure of the conidia to different atmospheres the percentage germination in 24 hours in part indicated merely a lengthening of the latent period, since in a number of the tested sets additional conidia germinated after the lapse of 24 hours. Here lengthening of the latent period is regarded first as giving *prima facie* evidence of weakening of the conidia.

In all atmospheres, with increased length of exposure to a given atmosphere, the percentage germination in 24 hours decreased at least with the longer exposures, beginning at 16 or 32 days on agar, and even at 8 days in water. With increased length of exposure the decline was either regular or irregular. In one case, however, the percentage germination was zero throughout.

This general decline is shown by Table VII, which records the approximate *average* percentage germination of conidia in 24 hours, in six sets of experiments in which the relative humidities of the storage atmospheres were 0 per cent. (H_2SO_4), 0 per cent. (P_2O_5) and 72.6 per cent., and the temperatures 20 and 30° C., the figures in columns 3-8 being obtained by averaging the data given in Table VI.

Table VII.

Average percentage germination.

Medium for germination	Temp. of exposure ° C.	Number of days exposure					
		1	(2) 3	8	16	31-43	64
Water	30	22	(18)	8	9	5	—
Water	20	24	(31)	14	10	3	—
Malt agar	30	43	28	23	7	2	—
Malt agar	20	79	82	68	73	48	22

Table VII illustrates the higher percentage of germination on agar frequently met with, and also suggests a tendency for exposure at 30° C. to have a more marked effect than that at 20° C. in depressing the percentage germination in 24 hours.

That intrinsic differences (*i.e.* differences before sowing) existed in the spores was suggested by the facts that: (1) the percentage germination of conidia was sometimes higher and sometimes lower on agar than in water, even when the treatment of the conidia was otherwise the same (apart from conceivable differences in position in the media which will be discussed later); (2) the percentage of conidia germinating, in water,

after the same treatment varied widely; (3) with increased duration of exposure to a given atmosphere there was sometimes a rise in the percentage germination.

The conidia exposed to air of 26 per cent. relative humidity showed higher percentages of germination on agar than the conidia exposed for the same periods to other humidities.

Under like circumstances there was no regular difference to be recognised between the effects of the very dry air (0 per cent.) and of the moist air (72.6 per cent.) on the percentage of germination.

Incidentally, these experiments proved that conidia can live after 136 days exposure to moist air (72.6 per cent.) at 20° C. and after 129 days exposure to air of 26 per cent. relative humidity at 20 or 30° C.

Germination in water subsequent to 24 hours submersion.

Table VI shows that exposure of the conidia to the same atmosphere for different numbers of days caused no corresponding change in the percentage of conidia germinating in water during various periods exceeding 24 hours. For instance, in these experiments the highest percentage germination obtained was 62 per cent. and that from conidia that had been previously exposed for 32 days at 20° C. to air of zero humidity (conc. H_2SO_4): conidia exposed to the same air for only 3 days gave a germination percentage of only 28 after the same number of hours in water, namely, 96.

Intrinsic differences among the conidia acquired before exposure to the atmospheres possibly concealed differences in behaviour induced by that exposure, and the same may be true in reference to the effect of the same length of exposure of the conidia to different atmospheres, as the percentages of germination showed no regularly corresponding difference.

It is true that no conidia germinated in water after exposure at 30° C. to air of 72.6 per cent. R.H.¹ for 32 days, or of 0 per cent. R.H. for 64 days, even when kept in water for 168 or 192 hours: in these cases weakness may have resulted in death, but this is not certain, as in another experiment some conidia first germinated after being in water for 192 hours.

(d) Variability in the behaviour of the conidia.

The lack of any regular agreement between the percentage of conidia (from cultures 3 days old) germinating within 24 hours or subsequently,

¹ Hereafter R.H. is used to denote the relative humidity of the air.

and the nature of the atmosphere and period of their exposure to it, suggests the intervention of some disturbing factor.

The disturbing factor or factors may have been acquired before the conidia were exposed to the various atmospheres, and may thus have been *intrinsic* differences; or they may have first come into being *during germination* and may have been mere differences in the conditions to which the conidia were exposed on or in the medium.

Considering first the second alternative, it is conceivable that differences in the latent period of germination or even in viability might be due to the position of the conidia in or on the medium. When the medium was water objections to this suggestion are the facts that: (1) no such difference in position was noted as all the spores were in focus under the microscope at the same time; (2) similar discords in the percentage germination were exhibited on the thin film of malt agar on which the conidia were lightly placed.

As regards differences in the conidia *before* exposure to the chosen atmospheres it is possible that these might be inherent and exist at the time of production or be induced by the slight differences of age (at most 2 days) and the consequent responses to the environment. Or the differences in the characters of the conidia might be due to external causes, including the direct or indirect action of the liquid excreted by the fungus, which would act differently on the spores according as these were in the water, near it, or more distant from it, and according as they were thereafter subjected to drying. That drying over calcium chloride could not wholly explain the anomalies in behaviour is proved by the fact that young conidia, germinated in water, showed similar anomalies although they were transferred straight from the culture to the water without any drying. In any case, whatever be the cause or causes, the young conidia used in our experiments, despite the relative uniformity of treatment, cannot be regarded as constant or uniform systems or material.

(e) *Atmospheric conditions lethal to the conidia.*

In view of the longevity of conidia lying on books and their consequent powers of initiating mildew, and in view of the possible utility of practicable methods of sterilising infected books, etc., by exposure to special atmospheres, a number of determinations were made of the times required to kill conidia exposed to atmospheres of different relative humidities at different temperatures.

Conidia were collected, from 3 days old cultures, on sterilised glass rods and exposed on these to different atmospheres ranging from 100 to 0 per cent. in constant relative humidity and from 100 to 1° C. at constant temperature. The relative humidities were obtained by means of sulphuric acid (A.R.) solutions the vapours from which were assumed to have no action on the conidia. After the requisite exposure the conidia were placed on 2 per cent. malt agar at 20° C. and were assumed to be dead if none of them germinated within 48 hours, as a number of trials showed that no germination of conidia took place on this medium after 48 hours.

Table VIII.
Time taken to kill the conidia.

Temp. ° C.	Relative humidity (%)												
	100	95-90	91	87-83	81	78	72.6	68	61	54	26	20	0
100	5 min.	—	10 min.	—	20 min.	—	20 min.	—	20 min.	25 min.	—	—	30 min.
73	—	—	—	—	1 hour	—	—	—	5½ hours	7 hours	—	24 hours	24 hours
52	2 hours	—	5 hours	—	17 hours	—	24 hours	—	2 days	3 days	—	V 20 days	10 days
45	17 hours	—	24 hours	—	24 hours	—	6 days	—	7 days	21 days	—	20 days	20 days
40	<38 days	—	—	—	—	—	14 days	—	V 22 days	18 days	—	V 22 days	18 days
35	G	G	G	—	72 hours	—	—	—	—	—	—	—	—
30	G	G	G	—	—	—	(32) days	—	—	—	(V 129) days	—	(71) days
25	G	G	G	G	G	<38 days	<38 days	<38 days	<38 days	<38 days	—	—	—
20	G	G	G	G	—	—	(V 136) days	—	—	—	(V 129) days	—	(V 64) days
15	G	G	G	G	—	—	—	—	—	—	—	—	—
10	G	G	G	G	—	—	—	—	—	—	—	—	—
8	G	—	G	—	V 32 days	—	—	—	—	—	—	—	V 25 days
1	G	—	G	—	—	—	—	—	—	—	—	—	—

Table VIII records the *observed* minimum times required to kill the conidia: it was not possible to make sufficiently frequent observations to determine the true minima. Most of the results given were obtained in this special group of experiments, but the results recorded in round brackets were obtained from the data given in Table VI. Where death of the spores did not occur within the period of the observations, the number of days of exposure to the given atmosphere is recorded in Table VIII together with the letter V denoting that the conidia were still viable. In a number of atmospheres the spores germinated on glass; these cases are indicated by the letter G. In some atmospheres, namely

those of 100 per cent. R.H. at 40° C. and 78–54 per cent. R.H. at 25° C., conidia tested for germination did not germinate on glass within 38 days. These were then placed on 2 per cent. malt agar at 20° C. and all excepting those exposed to 78 per cent. R.H. at 25° C. failed to germinate. Their death had occurred in less than 38 days, and this is indicated in the table by "<38 days."

The general results fall within those anticipated, as at temperatures above the maximum for germination in moist air, *i.e.* 40–100° C., there is in general a rise in the time required to kill the conidia with fall of temperature at the same relative humidity, and with fall of relative humidity at constant temperature. In detail there are minor discrepancies or irregularities, due in part to the limited frequency of observations and possibly in part to intrinsic differences in the conidia. Yet the data give sufficient idea of the order of magnitude of the combinations of time, temperature, and relative humidity that would be required to sterilise thin book materials and such-like infected with *P. chrysogenum*.

PART II. MILDEWING OF BOOK MATERIALS.

The germination in moist air of spores on glass is determined by two variable external factors, the relative humidity and the temperature of the air: the development of macroscopic mildew demands the introduction of a third external variable factor, a supply of appropriate nutrients.

The varied nature of the raw nutrient substances, namely, book materials, used in the present investigation introduces additional factors. These include: (1) the chemical nature of the various nutritive, inhibitory, and stimulating substances originally present or produced by the mildew fungus itself; (2) the effects of other organisms, fungi and bacteria, in the materials, in removing or producing water and useful or toxic substances (for reasons given later the book materials used were not sterilised); (3) an additional physical factor introduced by the hygroscopic nature of all book materials and by solutes present, which oppose resistance to the absorption of water by the mildew fungus; (4) during the progress of the investigation an additional factor revealed itself in the form of the water-excreting activity of *P. chrysogenum*.

The investigated materials used in the construction of books include untanned animal skins (vellum and parchment), tanned animal skins, finest silk gauze (for repairs of papers), and various vegetable products including binding cloth, paper (for printing and wrapping), millboard and strawboard. Samples of various kinds of these were supplied to us

by the Public Record Office and were used in our research. A list of the materials and some particulars concerning them are given in Appendix I.

The investigation covered two methods which differed in detail as indicated below.

(a) *First method.*

Small fragments, about $\frac{1}{2}$ in. square, of all the room-seasoned materials were placed on perforated zinc trays in airtight glass vessels, 4 in. in diameter, over various solutions causing the closed atmospheres to have definite constant relative humidities ranging from 100 to 61 per cent., namely, 100, 95, 91, 87, 83.5, 77 and 61.5 per cent.: the vessels and their contents were kept in ovens at constant temperatures of 10 or 15° C.

Bacteria and spores and mycelia of fungi occur on or inside all samples of book materials. If ordinary methods of sterilising materials by wet or dry heat had been employed the physical and chemical characters of the book materials would have been changed, and consequently it would have been impossible to determine the atmospheric conditions in which *normal* book materials become mildewed. Accordingly the book materials were subjected to no sterilisation process, except in the case of samples ultimately kept in an atmosphere of 87 per cent. R.H. at 15° C. which were dried for 2 days at 105° C.

In most of the atmospheres tested the samples were artificially supplied with conidia of *P. chrysogenum*; but to samples in two sets of experiments in two atmospheres of relative humidities 91 and 61.5 per cent. at 10° C. no spores were supplied: while samples in two other atmospheres of relative humidity 83.5 per cent. at 10 and 15° C. were supplied with conidia of *Aspergillus (niger?)*. Mildew was recorded as being present only when visible to the naked eye: no endeavour was made to determine microscopically the occurrence or non-occurrence of the germination of the spores.

Records were made of the water contents of the samples and of the times elapsing between the placing of the materials in the selected atmosphere and either the first appearance of mildew or the conclusion of the experiment. In most cases the room-seasoned samples were placed straight in the atmosphere in which they remained throughout the experiment; but those eventually kept in the atmosphere of 77 per cent. R.H. at 15° C. were previously exposed for 73 days to an atmosphere of 61.5 per cent. R.H. at 10° C., while those eventually in an atmosphere of 87 per cent. R.H. at 15° C. had a preliminary drying for 2 days at 105° C.

(b) Second method.

The number of materials investigated was reduced to thirteen, which included eleven used in the first method and representing all three main groups (untanned and tanned animal skins, and vegetable materials) as well as two new binding cloths (38 and 39 in Appendix I).

In general the procedure of the first method was adopted, but changes as regards detail were introduced: (1) the samples before being placed in the atmospheres were supplied with liquid water; (2) very young conidia were invariably used, and when mildew failed to develop, the samples were repeatedly supplied with new conidia; (3) the temperatures and relative humidities of the atmosphere tested were different from those used in the first method. Small portions (about $\frac{1}{2}$ in. square) of the materials were treated with distilled water which was spread over the surface, in quantities that were estimated on the basis of the first method to be appropriate to the respective atmospheres. The samples were then placed in the moist atmospheres until the aggregate weight of all the different samples in one atmosphere remained constant: after this they were infected with spores. In no case, except possibly one (material 12 in an atmosphere of 81 per cent. R.H. and temperature 25° C.), was water given in excess of the minimum amount known to cause mildew.

There were three different sets of treatments adopted. In one set, after addition of water, the samples were kept in their atmospheres (81, 78, 72, 68, 61 and 54 per cent. R.H.) for 24 weeks at laboratory temperature which fluctuated on observed days between 14.4 and 25.5° C. and may be regarded as mainly varying between 16 and 20° C. with an average of about 18° C. In the two other sets, after the addition of water the samples were kept in the five atmospheres, 78–54 per cent. R.H. at 3° C., in order to inhibit growth of moulds, until hygrometric equilibrium was established. Then one set was placed at 5° C. for 7 weeks, at laboratory temperature for the next 13 weeks and at 25° C. for the remaining 5 weeks. The last set was placed at 25° C. for 7 weeks, at laboratory temperature for the next 7 weeks and replaced at 25° C. for the following 5 weeks (all except those in atmosphere 81 per cent. R.H. which were not exposed to a temperature of 5° C., but, after the addition of water, were placed directly at 25° C.). After the initial infection with young spores, at most 3 (rarely 5 days old), reinfections always with young spores were made at approximately weekly intervals¹.

¹ A summary of the results obtained from the first and second methods is given in Appendix II.

(c) *Mildew from artificial and other infections.*

With one exception, in which a yellow species of *Aspergillus* appeared, the mildew that developed and was allowed to grow sufficiently long for identification proved to be *Penicillium* and probably always *P. chrysogenum*, and this was true whether the samples were artificially treated with conidia of *Penicillium* or *Aspergillus*, or subjected to no artificial infection. The impossibility of distinguishing between mildews developed through artificial and other infection in these investigations was illustrated by three sets of experiments in an atmosphere of 91 per cent. R.H. at 10° C., as in two of these the samples were treated with conidia of *Penicillium*, but in the third no spores were added: after 61 days in this atmosphere the percentage of samples showing mildew were respectively 22.9, 61 and 48.6.

(d) *Minimum relative humidity for mildew.*

The lowest relative humidities of the air at which mildew developed varied inversely with the temperature between 10 and 25° C., i.e. 83.5 per cent. at 10° C., 77 per cent. at 15° C. and 72.6 per cent. at laboratory temperature and at 25° C. No mildewing occurred at 5° C.

Table IX records the lowest relative humidities at which germination on glass and mildewing of book materials took place at various temperatures.

Table IX.

Minimum relative humidity for germination and mildewing.

	Temp. ° C.				
	10	15	Lab.	20	25
Germination	87	87	—	83.5	81
Mildewing	83.5	77	72.6	—	72.6

These results may be compared with those of Tomkins(4), obtained with other species of moulds on culture media, in suggesting that nutrients lower the minimum relative humidity at each temperature at which germination of certain fungal spores takes place. Our results however do not necessarily imply that a rise in temperature in all conditions favours mildew caused by *P. chrysogenum*; for instance with a limited number of spores available and a material relatively dry it is quite conceivable that a raised temperature might increase germination before the requisite supply of dissolved food material had become available so that the sporeling might die (see later). A number of materials did in fact become mildewed at lower relative humidities at 10 than at 15° C.

The different groups of materials differed as regards the minimum relative humidity of the atmosphere in which mildew developed, as is shown in Table X, in which 0 means that no mildew appeared on the materials in the atmospheres tested (at laboratory temperature and 25° C. only relative humidities 81-54 per cent. were used). When two numbers are separated by a dash (-) the latter number indicates the minimum relative humidity at which mildew developed on *any* of the materials of a group, the former, the minimum relative humidity at which *all* the materials of that group mildewed. N means that even in saturated air some of the materials did not mildew.

Table X.

Minimum relative humidity for mildewing.

Nature of material	Temp. ° C.			
	10	15	Lab.	25
(1) Untanned skins	100-91	100-83.5	0	0
(2) Leathers	91-83.5	95-77	78-72.6	78-72.6
(3) Vegetable	N-91	N-83.5	81-78	81-78
(a) Printing papers	N-91	N	0	0
(b) Binding cloths	N-91	100-83.5	81-78	81-78
(c) Binding boards	91	95	81	0
(d) Wrapping	91	95	0	0

Thus in all three groups mildew developed at 10° C. in air of 91 per cent. R.H. and at 15° C. in air of 83.5 per cent. R.H. At laboratory temperature mildew did not develop in group 1 at a relative humidity less than 83.5 per cent., but did develop in group 2 at 72.6-78 per cent. R.H., and in group 3 at 78-81 per cent. R.H. At all temperatures the lowest minimum relative humidity at which mildew developed was shown by the leathers. The various causes of failure to yield mildew are discussed later.

(e) "*Latent period of mildewing.*"

The time elapsing between the placing of the dry materials in their respective moist atmospheres, as described in the first method (p. 646), and the first appearance of mildew, is here termed the "latent period of mildewing," and is of practical significance as it gives some information on the time required for physically dry books in a room to become mildewed. This period ranged from a minimum of 5 days in a saturated atmosphere at 15° C. to 110 and 105 days respectively in atmospheres of 83.5 and 91 per cent. R.H. at a temperature of 10° C.

This period was much longer than the latent period of germination in the same nominal atmospheres, as the hygroscopic materials on the

one hand lowered the relative humidity of the air and on the other hand required time to become sufficiently moist to release their nutritive solutes. The latent period of mildewing generally varied directly with the relative humidity and temperature (10–15° C.).

In the second method (p. 647) the latent period of mildewing, measured from the time of the first application of conidia, varied from 4 weeks in the case of material 22 in an atmosphere of 78 per cent. R.H. at 25° C. to 24 weeks in the cases of materials 22 in an atmosphere of 72.6 per cent. R.H. and 19 and 29 in an atmosphere of 78 per cent. R.H. at laboratory temperature.

(f) *Liability to mildew.*

The liability to mildew varied with the nature of the materials, which can be ranged in the following order beginning with the most susceptible: (1) 22; (2) 29 and 19; (3) 12, 39, 25 and 33; (4) the remaining materials. The comparative susceptibility of the materials to mildew was shown by the minimum relative humidity at which mildew developed and the rapidity with which the material became mildewed. Material 22 alone developed mildew in 72.6 per cent. R.H. and was the first or among the first to do so in other atmospheres. Materials 29 and 19 (with 22) were the sole ones to mildew in air of 78 per cent. R.H. and usually were the second, or occasionally (with 22) the first, to show mildew. After these the materials becoming mildewed in air of 81 per cent. R.H. did so simultaneously with or subsequently to some or all of the three aforementioned.

In the cases of four of the materials the same correspondence between minimum relative humidity and length of the negative period for mildewing is indicated by comparison of the results obtained using the first and second methods. This is shown in Table XI, which

Table XI.
Number of weeks required for appearance of mildew.

		R.H. of air (%)														
		72.6			78			81			83.5		91	95	100	
Temp. ° C.	Lab.	5		25	25		25	25		25	10 15		10	15	10 15	
		Lab.	Lab.	Lab.	Lab.	Lab.	Lab.	Lab.	Lab.	25	10	15	10	15	10	15
22 (leather)	24	18	12		16	4	9	9		7	4	3	0.7	1.4	0.7	
19 (leather)	-	-	-		24	18	11	9		-	4	4	2.1	.	1.3	
29 (cloth)	-	-	-		24	20	9	9		-	15	8	2.1	1.4	0.7	
25 (cloth)	-	-	-		-	-	11	17		-	-	5	-	1.4	2.1	

incidentally illustrates that the negative period of mildewing lengthens with increasing dryness of the air, and with fall of temperature below 25° C., and records the minimum length of the negative periods of mildewing approximately in weeks: a dash (—) denotes no mildew; a dot, no test.

(g) *Water content.*

When moisture equilibrium between material and air is attained, at the same vapour pressure and temperature the percentage water content increases with the increasing hygroscopicity of the material. Accordingly it would be anticipated that when water is supplied to the material solely by the air the minimum percentage water content in a given atmosphere requisite for mildewing of different materials would vary directly with the hygroscopicities of these (and not with their respective water contents) unless disturbing factors (*e.g.* chemical differences) intervene. For the purpose of comparing the different materials, these are ranged into three groups: (1) untanned skins (parchment and vellum); (2) tanned skins (leathers); (3) binding cloths (vegetable fibres with size, etc.). Groups 1–3 represent a series declining in hygroscopicity, as is shown by: (i) the percentage water contents of the samples when received by us from the Public Record Office, and (ii) the percentage water contents of samples belonging to the three groups that had been exposed for 79 days to air of 61·5 per cent. R.H. at 10° C. The range and average of these water contents are given in Table XII.

Table XII.

Range and average of the water contents of materials (i) when received from the Public Record Office, (ii) after 79 days exposure to air of 61·5 per cent. R.H. at 10° C.

Group	Percentage water contents of materials			
	When received from P.R.O.		After 79 days exposure to air of 61·5 % R.H. at 10° C.	
	Range	Average	Range	Average
1	13·6–20·2	16·8	20·3–23·7	22·0
2	10·4–15·8	12·7	12·7–19·7	14·8
3	4·6–9·4	6·8	9·0–12·1	9·6

It will be noticed that the minima of groups 1 and 2 respectively exceed the maxima of groups 2 and 3.

The minimum water content at which mildew developed in the three

respective groups of materials varied directly with the hygroscopicity of the group for:

(i) Taking into consideration all the samples in all the atmospheres, the maximum water content acquired, and the minimum water content at which mildew developed in the three groups, varied in the same sense, as is shown in Table XIII which records the ranges and averages of these water contents (as estimated during the first method).

Table XIII.

The maximum water content acquired and the minimum water content necessary for mildew in all the atmospheres.

Group	Water content				Temp. ° C.
	Maximum		Minimum for mildew		
	Range	Average	Range	Average	
1	78.9-57.5	67.7	78.9-32.2	61.4	10
2	40.9-24.5	31.8	38.8-17.1	23.9	
3	28.7-18.4	24.9	14.9-10.0	12.9	
1	99.4-65.1	82.0	76.9-33.0	56.2	15
2	71.9-28.8	39.5	32.5-19.4	26.0	
3	44.6-19.6	27.6	44.6-10.8	18.6	

(ii) The percentages respectively of samples acquiring a maximum water content and first becoming mildewed at a water content exceeding 30 per cent. was greatest in group 1 and least in group 3 as is shown below in Table XIV.

Table XIV.

Percentage of samples acquiring a maximum water content and first becoming mildewed.

Group	10° C.		15° C.	
	Maximum %	Mildew %	Maximum %	Mildew %
1	100	100	100	100
2	37.5	25	75	12.5
3	0	0	33.3	11.1

(h) Absence of mildew and its causes.

The failure of mildew to develop on samples in these experiments was due to a variety of causes. Some of the failures, when conditions appeared favourable to the development of mildew, may possibly have been associated with lack of uniformity of one and the same material. For instance, the hygroscopicity of one small test sample of a material may have been higher than that of another, so that under the same

external conditions a minimum water content that sufficed for the mildewing of the latter sample might have been inadequate for that of the former; and the same might apply to cases where the two samples were in two atmospheres differing in relative humidity at the same temperature. Again the different small test samples may differ in the amount of toxic substances or in the abundance or nature of the competing organisms in the material.

Among the conceivable causes of failure to become mildewed, in addition to the few cases in which viable spores may not have been present, are: (1) lack of sufficient water available either in the air or in the material singly or in both together; (2) in the presence of sufficient water the failure was sometimes due not to lack of germination of the conidia but to arrested development of the sporeling. The possible causes of such arrest include lack of sufficient appropriate nutrient in solution, consumption of oxygen in or at the surface of the material, excess of toxins either originally present in the material or produced therein by bacteria and moulds.

In connection with suggestion (2) a small experiment was set up to show whether the spore germinated or not on the material. The two paper materials 7 and 10 failed to develop mildew at 10 or 15° C. in saturated air even after 77 days exposure at the lower temperature or 21 days exposure at the higher. Accordingly samples of these two materials were supplied with conidia and exposed to saturated air at 20 and 25° C. After 10 days the conidia had germinated on both materials, but although the samples remained in those atmospheres for 45 days material 10 did not become visibly mildewed.

(2) *Excretion of liquid water by P. chrysogenum.*

The vigorous excretion of drops of water by this species is recorded by Thom. Our cultures of this fungus, grown in Petri dishes at laboratory temperature, 20 and 25° C. on agar containing either Czapek's solution or 2 per cent. malt, habitually showed not only on the mycelium numerous macroscopic, colourless or pale yellow, aqueous drops, ranging in size up to a pin's head, but also countless, colourless, microscopic drops lying between the hyphae. As these drops were excreted into air that was not saturated (see the sequel) the fungus can thus raise the relative humidity of the air in a more or less confined space, and might directly or indirectly add to the water of the nutritive medium. However, this functional activity can play another part in spreading mildew, in that conidia can germinate in these excreted drops. For instance, on a

7 days old culture on 2 per cent. malt agar at 20° C. one excreted drop contained a small percentage (*circa* 1 per cent.) of germinated spores, some of which had produced branched hyphae. This same culture when 10 days old showed little pits in the surface each corresponding to one dried-up drop and containing a minute white tuft of mycelium.

Conidia dropped on films of 2 per cent. malt agar and kept in various atmospheres at 25° C. yielded the following results: in air of 91 per cent. R.H. after 3 days the mycelial growth resulting was blue-green with conidia, but showed no drops; after 4 days numerous microscopic drops occurred on the hyphae. In air of 78 per cent. R.H. after 3 days there was only a minute white mycelial growth showing no drops; after 7 days the macroscopic appearance was unchanged save that the agar showed evidence of drying and the microscope showed that the hyphae were swollen and distorted and that neither conidia nor even microscopic drops were present. In air of 54 per cent. R.H. even after 6 days although conidia had germinated no macroscopic mycelium had developed and the agar had dried.

A few additional experiments were made on the excretion of water drops by the fungus growing on book materials in a saturated atmosphere at 25° C. In this atmosphere materials 22 (goatskin) and 29 (buckram) yielded well-developed growths of various kinds of moulds without artificial infection or after infection with *P. chrysogenum*, but they produced no drops during the 10 days of the experiment. Accordingly samples of these two materials and of three others were sterilised in a steam steriliser at 100° C. for 40 min. and thereafter infected with conidia of *P. chrysogenum* and kept in saturated air at 25° C. After 4 days samples 22 and 29 both showed well-developed mycelia and no macroscopic drops, while the first had numerous and the second few microscopic drops. The other three materials, 11 (paper), 33 (millboard) and 37 (wrapping paper), yielded a poor growth of mildew in which no drops occurred.

The excretion of liquid water by the fungus probably played no significant part in the development of mildew in our general experiments on book materials, as the samples were freely exposed to air of constant temperature and relative humidity and any excess of water in contact with the air would speedily have been absorbed by the solution regulating the atmospheric humidity: moreover the observations were continued only until the mycelium first became visible. But in a library conditions are very different. The temperature and relative humidity of the general air fluctuate and, the latter especially, may differ considerably and for long periods in different parts of the room. Moreover the books are

largely pressed laterally against one another, so that the intervening atmosphere is reduced to a thin network or a collection of flat, more or less confined, little spaces, and if excreted water be due to respiration, fungal activity might directly increase the water content of the material as well as moisten the surrounding atmosphere.

SUMMARY.

(i) *Germination and viability of conidia of P. chrysogenum.*

1. Conidia were found to germinate in moist air on glass in atmospheres ranging in relative humidity from 100 to 81 per cent. and in temperature from 1 to above 35 but below 40° C., with the optimum temperature lying near 25° C.

2. At constant temperature the latent period of germination generally shortened as the relative humidity of the air rose, and when liquid or gaseous water was replaced by 2 per cent. malt agar.

3. The latent period of germination in water of conidia from old cultures (120 or 210 days old) tended to be longer than that of conidia from young cultures (3 days old). Variations in the intrinsic characters of the conidia caused irregularity in the results which was not shown when the conidia were germinated on 2 per cent. malt agar.

4. Conidia were exposed for periods up to 136 days to atmospheres of temperatures 20 and 30° C. and of relative humidities 72.6, 26 and 0 per cent. (approx.). The percentage germinations in water and on agar within 24 hours, and in water, after submersion for periods up to 216 hours, were determined. No correlation was found between the length of exposure of conidia to various atmospheres and the ultimate germination in water, though during the first 24 hours of germination on agar and in water increased length of exposure generally resulted in decline in percentage germination.

5. Exposure of conidia to atmospheres of 0–100 per cent. R.H. and 1–100° C. showed that conidia possess considerable powers of resistance to heat for temperatures up to 30° C. in atmospheres of relative humidity 0 and 26 per cent. and can live in the latter for more than 129 days; at temperatures above 40° C. the time required to kill the conidia at the same temperature falls with rise in relative humidity, and at the same relative humidity falls with rise in temperature.

(ii) *Mildewing of book materials.*

1. The 39 book materials investigated belong to three groups: (1) untanned skins (parchment and vellum); (2) leathers; (3) vegetable materials (binding cloths and boards, printing and wrapping papers). Samples were not sterilised in any way before investigation, except in a few isolated cases.

2. The conidia of *Penicillium chrysogenum* were, with few exceptions, used to infect the materials.

3. The constant relative humidities and temperatures used during this investigation ranged respectively from 100 to 54 per cent. and from 5 to 25° C.

4. At the same temperature the minimum relative humidities of the air necessary for mildewing of the materials were lower than for the germination of the conidia on glass. The lowest minimum for mildewing was 72.6 per cent. at laboratory temperature and at 25° C. No mildew developed at a temperature below 10° C.

5. It was found that groups 1, 2 and 3 represented a series declining both in hygroscopicity and in minimum water content requisite for development of mildew. At constant temperature the minimum relative humidity necessary for mildewing was lowest in the case of the leathers (group 2).

6. As the temperature rose from 10 to 25° C. the minimum relative humidity necessary for the development of mildew decreased and the "latent period of mildewing" (time elapsing between the placing of the dry materials in moist air and the first appearance of mildew) generally shortened with rise in relative humidity.

7. The excretion of drops of liquid by *P. chrysogenum* was the subject of some observations. In libraries it may help the spread of mildew, first directly, because conidia germinate in the drops, and secondly, by moistening the confined air between the mildewed sides of closely packed books.

For information concerning the composition of the materials other than parchment, vellum and leather, we are indebted to Messrs John Dickinson and Company, Ltd. (materials 5-11, 36 and 37); Messrs Ihlee and Sankey (material 34); The Winterbottom Book Cloth Company, Ltd. (materials 12, 24-30, 35) and Messrs Jackson's Millboard and Paper Company, Ltd. (materials 31-33).

Owing to the death of Prof. P. Groom, the junior author has finished

the compilation of this paper, and wishes here to express her gratitude to Prof. V. H. Blackman for his advice in preparing the paper for publication.

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APPENDIX I.

LIST OF BOOK MATERIALS OBTAINED FROM THE
PUBLIC RECORD OFFICE.No. *Untanned skins.*

1. Parchment. Late eighteenth century.
2. Parchment. Modern, used for repairs.
3. Vellum. Hide, ? seventeenth century.
4. Vellum. Modern, for binding.

Papers.

5. Linen fibre, sized with glue. Sixteenth or seventeenth century.
6. Linen fibre, a little later than 5. No colour.
7. Linen fibre, 1812, sized with glue, tinted with finely ground cobalt glass (smalt blue).
8. Linen fibre, 1848, sized with glue. No colour.
9. Linen rag, modern, hand-made, for repairing.
10. "Second" (guard paper). H.M.S.O. Modern. Chemically prepared wood-pulp, with traces of rag fibre. Sized with glue, natural colour.
11. Linen-backed (guard paper). Modern. Chemically prepared wood-pulp, sized with resin, and pasted with starch preparation to linen backing. No colour.

Linen cloth.

12. "Holland," modern, for mounting. Cotton or flax, sized with corn starch, corn flour, a little rice flour. Undyed.

Silk gauze.

13. Modern, for repairing.

No. *Binding leathers.*

14. Calf (flesh side out). Seventeenth or eighteenth century.
15. Calf (flesh side out). Nineteenth century.
16. Calf, ordinary, slightly sprinkled. Late eighteenth or nineteenth century.
17. Calf, coloured green. Modern.
18. Morocco (goatskin), coloured red, modern.
19. Pigskin, coloured (a) dark blue, (b) red, modern.
20. Pigskin, natural, modern. (Pure vegetable tanned.)
21. Niger goatskin, natural, modern.
22. Niger goatskin, native-coloured red, modern.
23. Seal, natural, modern.

Binding cloths.

(24-30, cotton or flax fibre, sized with corn starch, corn flour, with a little rice flour.)

24. Cloth, watered, dyed (a) dark green, (b) dark reddish purple, modern.
25. Cloth, plain, dyed (a) red, (b) dark reddish purple, modern.
26. Cloth, morocco grain, dyed red, modern.
27. Buckram, linen, white, modern.
28. Buckram, linen, dyed scarlet, modern.
29. Buckram, cotton, white, modern.
30. Art canvas, dyed red, modern.

Binding boards.

(31-33, the fibre used is old paper, and may contain any class of vegetable fibre.)

31. Millboard, old, rough surface.
32. Millboard, modern, smooth surface.
33. Millboard, modern, smooth surface, thin.
34. Strawboard, Dutch, modern rough surface. Fibre is straw of ordinary wheat, rye, barley or oats, probably a mixture of two or more of these. No size. Lime is mixed with the straw, and the pulp boiled. Sheets pasted together with water-glass.

Wrapping.

35. Glazed cloth, modern. Fibre is cotton or flax, size is corn starch, corn flour with a little rice flour.
36. Brown paper, old. Raw flax fibre, probably flax waste associated with the lignified woody tissue of the flax plant. Natural colour.

No.

37. Mill wrapping paper, modern. Fibre chemically prepared wood pulp and jute, with a small proportion of straw and mechanical wood pulp. Dye, aniline blue.
38. Binding cloth, very similar to 25, scarlet.
39. Binding cloth, very similar to 26, scarlet, small grain.

Group 1. Materials 1-4.

Group 2. Materials 14-23.

Group 3. Materials 5-12, 24-37, 38-39.

(Material 13, being unique, was not included in any of the groups.)

APPENDIX II.

Data concerning

A. The time taken for mildew to develop on

B. Occurrence or non-occurrence of mildew on

C. Average percentage water content of

the three groups of materials in twenty-six different atmospheres.

In Table XV below, column A records the number of days required by the members of a group to become mildewed; column B represents the occurrence (x) of mildew on one or more members or non-occurrence (0) of mildew on all the members of a group; column C gives the average percentage water content of *all* the members of a group in the atmosphere under consideration, whether mildewed or not.

Table XV.

Group	A	B	C	A	B	C	A	B	C
	100 % R.H. 15° C.			100 % R.H. 10° C.					
1	7-21	x	81.9	21-74	x	67.7			
2	5-15	x	44.6	10-26	x	30.5			
3	5-21	x	31.2	6-74	x	27.1			
	95 % R.H. 15° C.			91 % R.H. 10° C.					
1	22	x	47.6	28	x	38.5			
2	5-15	x	32.0	21-53	x	23.9			
3	5-22	x	19.5	21-80	x	13.9			
	87 % R.H. 15° C.			83.5 % R.H. 15° C.			83.5 % R.H. 10° C.		
1	60	x	—	71	x	30.3	—	0	35.5
2	28-60	x	—	28-102	x	20.6	50-110	x	21.2
3	38-60	x	—	65-102	x	11.1	—	0	11.6
	81 % R.H. 25°-lab.-25°			81 % R.H. lab.					
1	—	—	—	—	—	—			
2	61	x	19.7	61-75	x	20.0			
3	61-122	x	12.2	61-75	x	11.2			

Table XV (cont.).

Group	A	B	C	A	B	C	A	B	C
	78 % R.H. 25°-lab.-25°			78 % R.H. 5°-lab.-25°			78 % R.H. lab.		
1	-	0	32.0	-	0	31.3	-	0	30.2
2	28-130	x	17.0	155	x	18.5	112-171	x	19.5
3	146	x	10.8	-	0	10.0	171	x	9.9
	77 % R.H. 15° C.								
1	-	0	-						
2	48	x	-						
3	-	0	-						
	72.6 % R.H. 25°-lab.-25°			72.6 % R.H. 5°-lab.-25°			72.6 % R.H. lab.		
1	-	0	30.0	-	0	28.5	-	0	28.6
2	112	x	15.2	124	x	15.5	171	x	15.9
3	-	0	9.7	-	0	8.5	-	0	8.9
	68 % R.H. 25°-lab.-25°			68 % R.H. 5°-lab.-25°			68 % R.H. lab.		
1	-	0	27.6	-	0	25.6	-	0	26.1
2	-	0	14.9	-	0	13.6	-	0	14.5
3	-	0	8.6	-	0	9.6	-	0	8.1
	61.5 % R.H. 25°-lab.-25°			61.5 % R.H. 5°-lab.-25°					
1	-	0	22.6	-	0	23.5			
2	-	0	11.2	-	0	14.8			
3	-	0	6.9	-	0	7.4			
	61.5 % R.H. lab.			61.5 % R.H. 10° C.					
1	-	0	20.9	-	0	22.0			
2	-	0	12.9	-	0	14.8			
3	-	0	5.8	-	0	9.7			
	54 % R.H. 25°-lab.-25°			54 % R.H. 5°-lab.-25°			54 % R.H. lab.		
1	-	0	23.2	-	0	24.7	-	0	18.9
2	-	0	12.6	-	0	12.9	-	0	12.1
3	-	0	7.5	-	0	7.9	-	0	4.6

R.H.=relative humidity.

In the above table, lab.=the temperature of the laboratory, which varied between 14.4 and 25.5° C., and may be regarded as having an average of 18° C.

25°-lab.-25° and 5°-lab.-25° mean that the materials were first placed for some time either at 25 or at 5° C., then kept at laboratory temperature and finally kept at 25° C.

A (-) indicates either that no data are available (in the case of occurrence of mildew or percentage water content) or, in the case of column A, that for the duration of the experiment no mildew was observed on any members of a group.

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CHANGES IN THE CELL CONTENTS OF WOOD (XYLEM PARENCHYMA) AND THEIR RELATIONSHIPS TO THE RESPIRATION OF WOOD AND ITS RESISTANCE TO *LYCTUS* ATTACK AND TO FUNGAL INVASION

By S. E. WILSON, M.Sc., Ph.D., D.I.C.
(*Royal Veterinary College, London.*)

(With 11 Text-figures.)

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PART I.

INTRODUCTION.

THE reserve food materials stored in the stem of the tree have been studied by numerous observers during the last seventy-five years. Chief among these substances are starch and fat, and trees have been classified according to the preponderance in the sapwood of one or the other substance. Büsgen⁽²⁾ has summarised and discussed the facts recorded down to 1926 on the occurrence of reserve substances in the living tree. He remarks on the "want of harmony among the existing data," explaining it as being partly due to a difference of behaviour between young twigs and the stem, and partly to a difference in chemistry between the various

species. All the observers referred to by Büsgen agree, however, that reserve materials accumulate in the sapwood (*i.e.* in the xylem parenchyma, including the medullary rays) during summer, and remain, with certain modifications, until spring, when they are drawn upon for the new growth of the tree. Starch, when present, is at a maximum just prior to leaf-burst. Cockerham (5) has recently investigated the relative starch content, round the year, of different regions of the sycamore tree, and has shown that abundant starch is present in the trunk of the tree from late summer onwards to March, with minor variations.

No observations appear to have been recorded, however, on the fate of these substances after the felling of the tree, *i.e.* their behaviour in *timber*. This is somewhat surprising in view of the widely known liability of sapwood (as timber) to infestation by invading organisms, particularly insects and fungi. So great have been the losses sustained in this way that the finding of methods of control became an urgent necessity. As a result, a good many investigations have been made, but such as have been recorded have dealt almost entirely with the physical, rather than the biological, aspects of the problem. Indeed, wood has seemingly been regarded solely as ligno-cellulose, while the cell contents, except for water, have been conspicuously ignored. In the present paper the importance of cell contents will be demonstrated, and particular emphasis laid on *starch*, which is the most frequently occurring reserve substance in the sapwood of timber trees.

STARCH DEPLETION IN EXPERIMENTAL PIECES OF SAPWOOD.

Since the starch content of a tree varies according to the species and the time of year, it follows that in the case of felled timber the amount of starch in the sapwood depends upon the species and the time of year at which the tree is felled. That the quantity then present, however, remains thereafter constant is by no means the case, as was discovered by the experiment here described.

Short lengths of branches were cut from ash, oak and other trees in July. The pieces, about 2 in. in diameter and $\frac{3}{4}$ in. in length, with the bark on, were dissected while fresh by chopping out a segment as shown in Fig. 1. A small amount of starch was observed to be present distributed throughout the sapwood. After they had been left at room temperature for six months the specimens appeared quite dry, and it was found that striking changes had taken place in their starch content.

In the piece of ash, for example, all the surfaces which had originally been exposed (always excepting, of course, the bark), which are here

called "original surfaces," were coloured uniformly blue when tested with iodine solution, indicating little, if any, change at the surface. Further

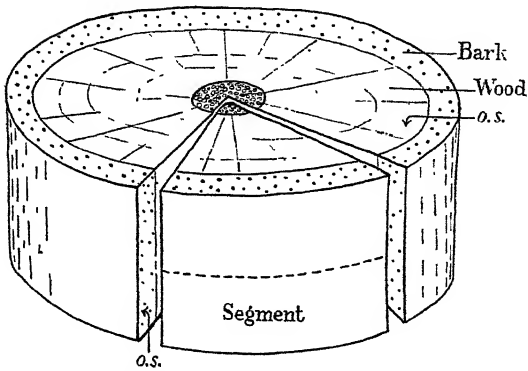


Fig. 1. Disc cut from branch of ash tree. *o.s.*, original surface.

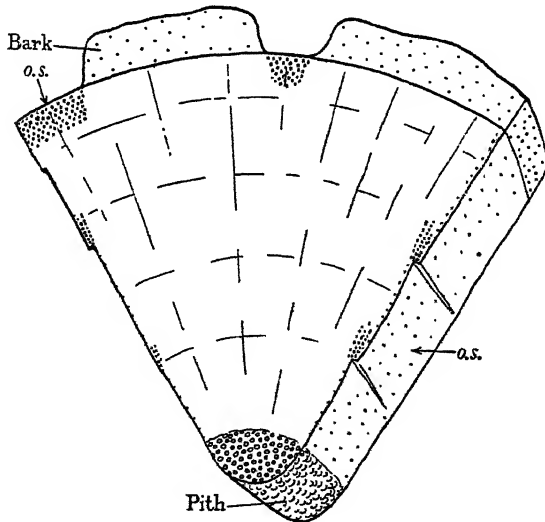


Fig. 2. Segment of dry ash disc, after cutting across at the broken line in Fig. 1. Red indicates starch. Note patches of dense starch where bark had been removed while the wood was fresh. *o.s.*, original surface.

dissection of the disc revealed the almost complete disappearance of starch from the main mass of wood.

Fig. 2 shows the starch distribution in the segment of the dry ash disc, as seen by cutting the piece across at the place shown by the broken line

in Fig. 1. At the radial edges of the newly cut transverse face of the segment there was a thin line of starch-bearing cells (original surfaces) from the bark to the pith. The medullary rays, whose ends were exposed at the jagged edges of the segment, contained starch for some distance along the rays, and "patches" of starch appeared in the youngest wood where the bark was missing. A radial longitudinal section through the disc, extending from bark to pith, showed that starch was present only in a shallow zone at the original surfaces of the disc (Fig. 3).

A similar disc of oak, after identical treatment, was found when dry to have in the sapwood a starch content generally similar to that of the

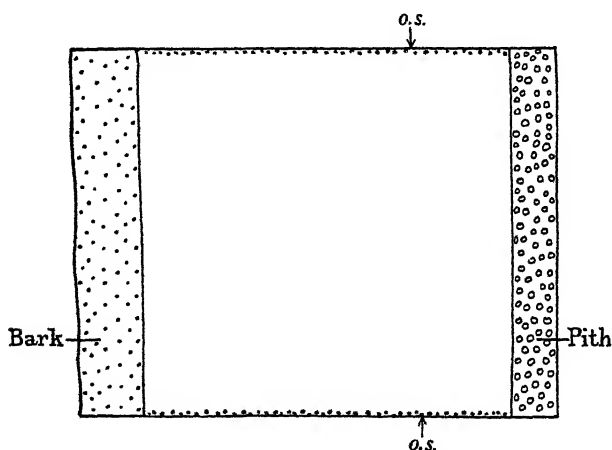


Fig. 3. Radial longitudinal section of dry ash disc.
Starch only at original surfaces.

ash disc, though, of course, the heartwood was free from starch. In this sample it was noted that where the bark was missing starch occurred to a greater distance inwards from the cambial face than where the bark was only slightly parted from the wood (Fig. 4). Sycamore, beech, sweet chestnut and elm also showed starch depletion in the same way but in varying degrees.

The result arrived at was that the original starch (no great amount) had more or less completely disappeared from the inner regions of the wood, and it seemed reasonable to conclude that the cells, alive when the sample was removed from the tree, continued to live and so depleted their reserve starch. On the other hand it was conjectured that as the starch had remained unchanged in the parts most exposed, the cells there had

died quickly, as a result of either mutilation or water loss, and had thus retained their original starch.

These results, which showed that starch depletion occurred naturally in felled timber, also indicated that there was an important relation between quick drying and starch retention, and between slow drying and starch depletion, or, in other words, that the *history of starch in timber was closely bound up with methods of seasoning*. This hypothesis formed the basis of further experiment.

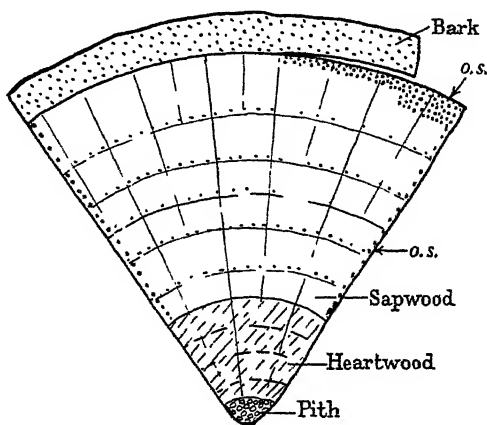


Fig. 4. Transverse section of segment of oak disc. Note depth of starch zone in relation to removal of bark.

METHODS OF SEASONING.

We have seen that experiments with small branches cut in summer pointed to a connection between the rate of drying and the amount of starch left in the wood. In order to examine this point further, the effect of different rates of drying was investigated.

A young ash tree, all sapwood, was felled in September, and successive lengths of the stem, which was about 4 in. in diameter, were treated in different ways, in accordance with the various methods usually adopted with ash timber in commercial practice. Thus a 6 in. length of the trunk was split into sticks, and, at room temperature, was dried quickly by means of an air stream from an electric fan, the process lasting several days. A second length was also cut up and the pieces steamed for 2 days, while a third lot of sticks were oven dried.

On examination it was found that no change had taken place in the quantity of starch in any of the three cases. All the wood treated was then

kept in the laboratory for a year, and there was still no change in the amount of starch. Some of the "air-dried" sticks (the first of the three cases) were then put through a normal kiln-seasoning process, with still no effect on the starch content.

These results supported the hypothesis that the sudden death of the cells (alive when the wood was felled), which took place either by desiccation or excessive heat, resulted in the retention of the whole starch

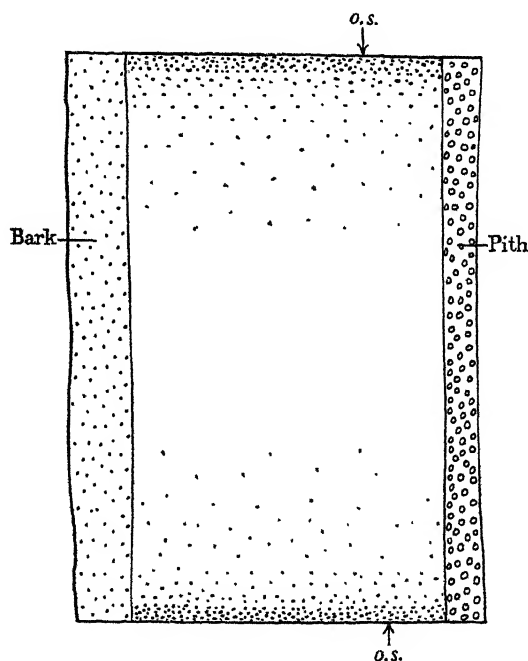


Fig. 5. Radial longitudinal section of a 4 in. length of dry ash branch.
Note dense starch at ends, diminishing inwards.

content, which, after the death of the cells, was not affected by further seasoning treatment.

The results were different, however, when the drying of the wood was retarded. A short length of the same ash stem, a disc measuring $1\frac{1}{2}$ in. along the grain, with the bark on, was placed in a cupboard, where it would be expected to dry at a moderate rate. After twelve months it was dissected and tested for starch by the microscopic examination of thin sections. Starch was found to be abundant near the original surfaces to a depth of $\frac{1}{2}$ cm., but it gradually decreased inwards until there was only a

small amount left in the middle. A 4 in. length of the same ash stem, after the same treatment, showed a similar starch distribution, except that in this longer piece there was still less starch left in the middle (Fig. 5).

These two cases illustrated the partial depletion of starch which resulted from delayed drying, but while starch depletion was the more complete the greater the distance from the original surfaces, yet these original surfaces always presented zones of abundant starch.

The next experiment to be described showed the effect of sealing the end surfaces in order to delay drying still further. A length of the same ash stem as before, with the bark intact, was sealed at both ends by the application of vaseline followed by melted paraffin wax. After fourteen months¹ the piece of wood was found on microscopical examination to be entirely devoid of starch throughout its length. A similar piece of the same ash tree was immersed in fresh water for the same period, with the same result.

These experiments served to show that starch could be completely eliminated from sapwood by ensuring delayed drying to all parts of the log, and that this delay must be sufficient to enable cell activity to continue long enough to exhaust all starch reserves.

FACTORS INFLUENCING THE TIME REQUIRED FOR STARCH DEPLETION.

It has been shown in the foregoing pages that if the drying of a log of wood (with the bark intact) is delayed by sealing the ends, starch-free sapwood will eventually result. The period of time required for this process will obviously depend upon a variety of factors, notably the species of the tree, the dimensions of the log, the initial amount and distribution of the starch, and weather conditions. Further information as to the process of starch depletion was sought by continuing the experimental study of small samples of timber, the results being then correlated with facts observed in the study of timber of commercial dimensions.

In the earlier experiment the sealed log of ash was not opened until starch depletion was complete, so that it was not known whether the depletion proceeded uniformly or not. A branch which was all sapwood and contained abundant starch throughout was cut from an ash tree in March. This branch, about 3 in. in diameter, was then cut into lengths of about 3 in. The ends were sealed with vaseline and wax, and the blocks hung in the laboratory. After a month one of the sample blocks was cut across and tested for starch. Three zones could be

¹ How the required period was determined is described later.

distinguished: (1) a peripheral zone of starch-free wood, $\frac{1}{4}$ in. wide, next the bark; (2) an intermediate zone of about the same width containing starch, the density increasing inwards; (3) a central zone where no appreciable diminution of starch had occurred (Fig. 6 *a*).

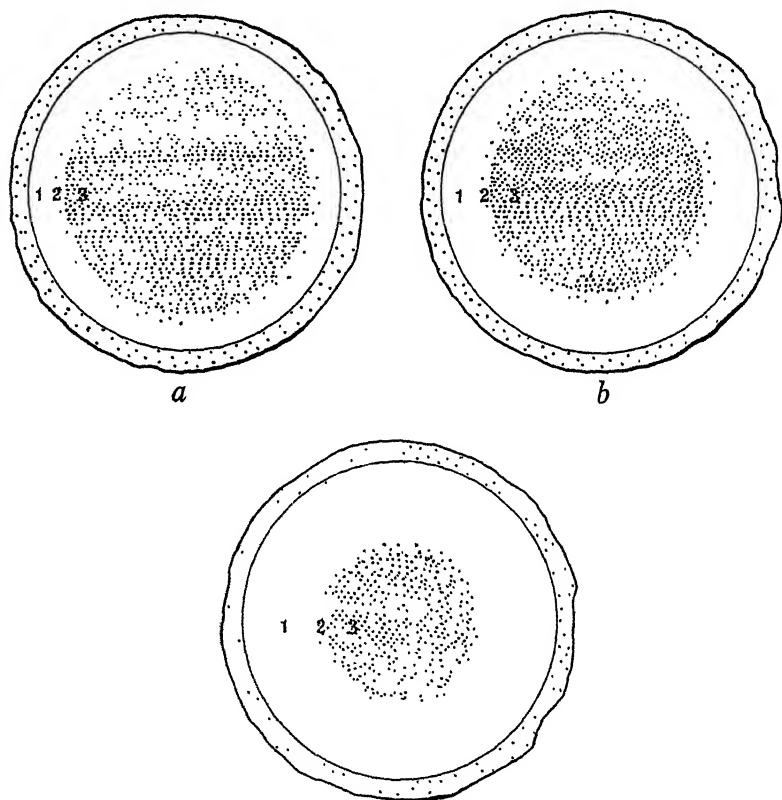


Fig. 6. Transverse sections of ash branch: *a* one month, *b* two months, *c* four months after removal from tree. Zone 1, starch depletion complete; zone 2, depletion partial; zone 3, no change.

A month later another block was examined and the outer starch-free zone was found to be not quite double the first in width, the intermediate zone being the same width as before, and the central zone accordingly smaller (Fig. 6 *b*). After a further two months a section of a third block showed that further depletion had taken place on the same lines, but that the rate was slower than in the previous months (Fig. 6 *c*). A slight

reduction of the amount of starch in the central zone was also apparent at this stage.

The conclusion was drawn that starch depletion does not occur uniformly throughout a log, but, in the main, takes place radially from the bark inwards, and that the greater the distance from the bark the slower the rate of progress. Hence it is obvious that the width of the sapwood in a log is one of the prime factors influencing the time required for the process.

Indeed, all the evidence goes to show that starch depletion is due to the respiration of living cells, the necessary gaseous exchange taking place through the bark. This being so, it appeared that the measuring of the respiratory rate would give relative data for tracing the effect of temperature on the rate of starch depletion in experimental samples.

Before considering the temperature factor in this way, it may be noted that there are two processes proceeding concurrently in these depletion experiments, both involving gaseous exchange, viz. desiccation (loss of water vapour), and respiration (with consequent starch depletion). Either process results ultimately in the death of the cells in felled timber. Thus, near the ends of an unsealed log death by desiccation (the water vapour travelling along the grain of the wood to the ends) supervenes before starch depletion by respiration is far advanced, and the final starch distribution is as shown in Fig. 5. In an end-sealed log on the other hand, the movement of water vapour across the grain of the wood and out through the bark, though it results in ultimate desiccation, is not sufficiently rapid to overtake starch depletion, and the cells therefore die by exhaustion of their reserve food substances.

The effect of temperature on the time required for seasoning may now be considered. By means of gas analysis it is a fairly simple matter to measure the respiratory exchange of any living organism of convenient size, since (aerobic) respiration involves the oxidation of carbon compounds with the consequent production of carbon dioxide.

At an earlier stage in this investigation it was desired to discover whether a block of ash sapwood (the subject of the experiment in starch depletion referred to on p. 667) was still respiring six months after being cut from the tree and the ends sealed. The block, comprising a litre of wood plus the bark, was placed under a bell-jar which had a capacity of 3.4 litres and was fitted with a tap. The bell-jar was sealed down with vaseline to a glass plate. After 3 days a sample of the enclosed atmosphere was drawn off at the tap into a vacuum tube and analysed by means of the Haldane apparatus. It was found that the carbon dioxide had risen to

5.7 per cent., while the oxygen concentration had fallen to 15.1 per cent. This represented a yield of 131 c.c. of carbon dioxide per litre of wood, with a consumption of 135 c.c. of oxygen. Two days later the carbon dioxide had risen further to 7.5 per cent. (The block of wood was superficially dry and was free from moulds.) It was concluded therefore that the xylem parenchyma was still respiring, although half a year had elapsed since the tree was felled.

The same block of ash was similarly tested at intervals of a few weeks and the rate of respiration found to be falling slowly. Thus eleven months after felling, the reading, taken as described above, for a 4-day period was 2.3 per cent. of carbon dioxide; finally, after a total period of fourteen months, the yield of carbon dioxide was nil. Respiration had ceased, and on microscopical examination no starch was found.

Oven-dried pieces and steamed pieces of ash from the same tree, and containing abundant starch, were tested at the same time, giving zero readings for carbon dioxide.

The gas-analysis method was next used for finding the (relative) effect of temperature on the respiratory rate of wood. This time oak sapwood was used. An examination of an oak tree at the time of felling had provided a branch $4\frac{1}{2}$ in. in diameter, which was all sapwood. An 11 in. length of this branch had been hanging in the laboratory, with the bark intact and the ends sealed, for about six months at the date of this experiment. The block was enclosed within a bell-jar as before, and at room temperature the wood was found to be slowly respiring. The block was placed in an ice-chest and allowed to cool down for 3 days. Carbon dioxide readings were then taken and the respiratory rate was found to have dropped considerably. In the same manner readings were taken for a temperature of 37°C . by utilising a boiler room, and the rate was found to be much higher. The total capacity of the bell-jar was 4500 c.c. and was occupied thus: oak sapwood 2000 c.c., plus bark 200 c.c.; air 2300 c.c.

Detailed results are as follows:

Temp. $^{\circ}\text{C}$.	Period	Yield of CO_2 in c.c. per litre of sapwood
19	24 hours	30
	2 days	65
	4 days	125
5	24 hours	17
	2 days	32.5
	3 days	43
37-39	24 hours	113
	2 days	162

In this experiment the range of temperature corresponded roughly to that of our climate, and the not unexpected result was that respiration,

and hence starch depletion, proceeded more rapidly at summer temperature than at winter temperature.

So far only small experimental samples of wood have been discussed, but in a timber log¹ of commercial dimensions, all the wood except a shallow zone at each end would be expected to behave like the short end-sealed blocks of the experiments on p. 668, while the "sticks" of the experiments on p. 665 may be regarded as corresponding to "converted" timber (*i.e.* logs sawn into commercial dimensions). Accordingly we may now turn to timber of commercial dimensions, and consider it in the light of all the factors affecting starch depletion which have so far emerged.

STARCH DEPLETION IN COMMERCIAL TIMBERS.

Several years' association with the English timber trade, while maintaining close contact with a biological laboratory, provided the writer with opportunities for tracing the history of starch in timber of commercial dimensions from the felling of the tree to the marketing of the finished product. Many of the common species of timber trees were found to agree closely with sycamore as described by Cockerham(5). Thus ash, oak, elm, beech, walnut and sweet chestnut were found to have a large amount of starch in the sapwood when the trees were felled between October and April, *i.e.* in the usual felling season for "hardwoods." In normal practice, the time during which felled timber stays "in the round" (*i.e.* in log form) varies from a few days to perhaps several years, depending on the kind of timber and the market demand, as also on the exigencies of extraction and conversion operations, *viz.* the hauling of the timber and the sawing into dimension stock.

It was found that in all cases when the logs were converted (sawn) shortly after felling and the sapwood treated in any way calculated to cause the early death of the xylem parenchyma, the initial quantity of starch remained in the sapwood, and was unaltered by any subsequent treatment. For example, young ash trees, 10-12 in. in diameter (all sapwood), were felled in February, and within a fortnight were converted and the wood steamed for bending into tennis racquet frames. Tests showed abundant starch in all the finished stock. The steaming (for enrichment of colour) of freshly cut beech blocks, from which shoe-lasts and boot-trees were to be made, likewise resulted in a finished product in which starch was plentiful. The kiln drying, as also the rapid air drying, of boards cut from freshly felled logs of oak, elm, sweet chestnut, beech and sycamore,

¹ The retention of the bark is implied in the use of the term "log" hereafter.

all for furniture or similar uses, were observed to leave the sapwood in its original state as to starch content.

On the other hand, it was found that the starch had completely disappeared from young ash logs which had been stored under cover for twelve months before conversion. Careful examination of such logs at intervals during the year showed that the starch depletion took place gradually, from the bark inwards, and more rapidly in the first four or five months than later.

The time required for starch to be cleared from oak logs felled in March was found to be proportional to the width of the sapwood in different regions of the same tree. A typical example was provided by an oak log which was some 40 ft. in length, 3 ft. in diameter at the base with sapwood $1\frac{1}{4}$ in. wide, and 14 in. in diameter at the top with sapwood $2\frac{1}{2}$ in. wide. In four months the starch had gone from the base of the tree, whereas eight months were required for complete depletion of starch in the sapwood double the width at the top end of the log.

The time schedule for starch depletion was different, however, in the case of trees felled in late November, for it was noticed that no marked change in the starch content occurred during two months of cold weather, but that the rate of depletion increased thereafter to a maximum in May and June. The retarding effect of low temperatures was found to apply to all widths of sapwood.

No observations were made on sycamore and beech logs as it is customary to convert these species as speedily as possible to avoid discoloration, which occurs if the timber is left "in the round," especially in warm weather.

The results obtained from an experimental study of small pieces of wood, as described earlier in this paper, were thus found to be paralleled in commercial timbers as far as the complete retention and the *complete* depletion of starch were concerned. *Partial* starch depletion remains to be considered.

Mention has already been made of the very variable periods during which timber remains in log form before being broken up, ranging from a few days to several years. Whereas the felling of "hardwood" trees is undertaken only in winter (the greatest activity taking place in the first quarter of the year), saw-milling goes on all the year round, so that although some "hardwood" timber is converted immediately, with full starch content, the major part must be converted while the sapwood is in a condition of partial starch depletion.

Now depletion will not necessarily cease entirely on the breaking up of

the log, for such sapwood as is "alive" at that time will continue to lose its starch unless exposed to quick drying or some other lethal factor. Thus treatment such as kiln drying, steaming or rapid air drying will have the effect of perpetuating the starch content, whether full or partial, as at the date of conversion; but slow drying at normal temperatures will result in continued starch depletion, for example, in the inside region of a thick plank. It must be remembered that all newly exposed surfaces come under the heading of "quick drying," and will therefore retain the starch content they possessed at the time of conversion.

For instance, an ash log was planked into various thicknesses shortly after felling, when the starch content was therefore high. A year and a

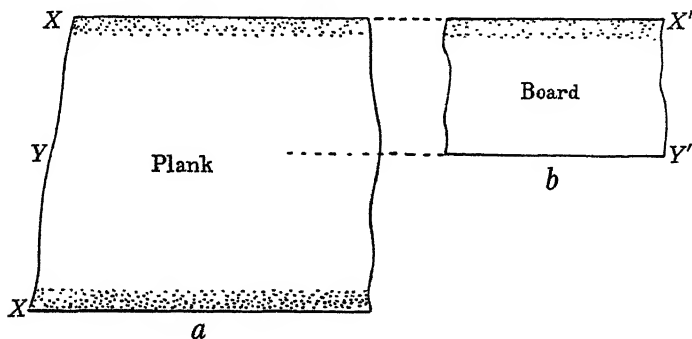


Fig. 7. *a*, ash plank 3 in. thick, all sapwood, sawn while fresh, then air seasoned. Note dense starch at both faces *X*, and absence of starch from middle region *Y*. *b*, a board "re-sawn" from the plank after seasoning. Note starch at beard face *X'*, while face *Y'* is free of starch.

half later it was found that a thin plank ($1\frac{1}{2}$ in.) contained practically the original amount of starch throughout, while a 3 in. plank showed surface zones of dense starch with a diminishing quantity inwards, and practically none midway between the two plank faces (Fig. 7*a*). In a 5 in. plank all the sapwood except the surface zones was without starch.

Thus, while it is true that gradually seasoned planks 3 in. or more in thickness, from freshly felled or from partially depleted logs, become for the most part free from starch, a zone of abundant starch varying in thickness from, say, $\frac{1}{8}$ to $\frac{1}{4}$ in., must inevitably remain at all exposed surfaces.

It is, of course, quite possible that when a log already partially depleted is broken up, some of the converted portions will be entirely free of starch at the time of conversion, but one can never say that *all* the

converted portions of such a log will be free of starch, even though they are seasoned gradually, because of the retention of starch by newly exposed surfaces.

It may be suggested that the iodine test would serve to distinguish between starch-free and starch-containing timber from logs only partly depleted, but experiments show that the starch distribution may become too complex for the test to be practicable. That this is so may be seen by taking an example of a plank (Fig. 7 *a*) from a log converted while fresh and then naturally seasoned; such a plank, *XYX*, has a starch zone at each surface and is free of starch in the middle. If, however, a board, *X'Y'* (Fig. 7 *b*), is "re-sawn" from the plank, it will have dense starch on one face, *X'*, and no starch on the other face, *Y'*, so that an iodine test on any one face of the board would be misleading.

Starch distribution becomes even more complicated when an ash log, all sapwood, is already partially depleted prior to planking (Fig. 8 *a*), and is afterwards naturally seasoned in the plank. At the time of conversion, an outer plank, *A*, is starch-free, while an inner plank, *BCB*, has a zone of starch in the middle which extends from face to face, and each edge (near the bark) is starch-free from face to face (Fig. 8 *b*). If this same plank is naturally seasoned the final stages will be that the starch will have gone from all but the faces of the middle width (Fig. 8 *c*). The iodine test is thus seen to be quite impracticable in converted timber from partially depleted logs, as the test applied at one part is not an indication of the starch content of the whole piece, nor is the starch content of the interior necessarily that indicated by the surface.

SEASONING BY IMMERSION IN WATER.

Immersion in water as a method of seasoning is not often practicable in this country, though it frequently occurs in other countries, where it is often incidental to the transportation of logs by floating. The effect of immersion on starch content was tested experimentally with small blocks of (converted) ash and oak sapwood, which were suspended while fresh felled in a supply cistern of running water, and examined at intervals. It was found that starch disappeared slowly from the xylem parenchyma, at much the same rate as in a sealed log in air. The amount of oxygen in the water was not estimated, but was probably lower than in a stream.

Logs of freshly felled ash and oak were kept in a pond for a year, by which time the starch had gone from the oak, the ash taking longer as the sapwood was wider. A further experiment was made to discover whether any change took place in the starch content of dried wood when re-

wetted. Blocks of dry ash and oak sapwood containing a good deal of starch were placed in water from a cistern, as above. After 4 years the

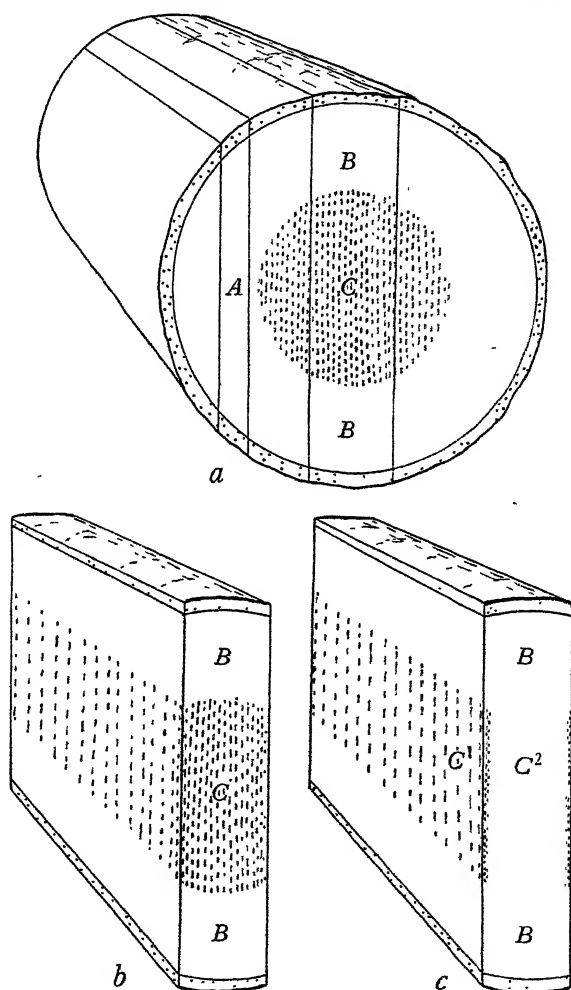


Fig. 8. *a*, ash log when partial starch depletion has occurred. *A*, outer plank free of starch; *BCB*, middle plank with starch at *C* and none at *B*. *b*, plank *BCB* exposed. Note starch distribution at time of planking. *c*, the same plank after further air seasoning. Note starch remaining at plank face *C¹*, the region between the surface zones being now free of starch, *C²*. N.B. The near end of this plank has been cut back say 1 in. to display the actual starch distribution.

full original amount of starch was still there in all the sound wood (the outside was by this time decayed).

There is room for doubt as to whether the starch depletion observed in timber immersed in water is to be explained by the respiration of the living cells. Depletion may possibly be due to the action of free enzymes. It is noteworthy, however, that the process, whatever it is, is not re-activated by re-wetting the wood after the death of the cells by desiccation. There is so far no evidence that the cells are killed by immersion; their survival, however, would raise the problem of an adequate supply of oxygen.

From the foregoing observations on timber of commercial dimensions certain main facts have emerged: firstly, the factors affecting starch depletion in logs are the initial quantity of starch due to the species and the time of felling, the width of the sapwood, and temperature; secondly, only log seasoning for a sufficient period of time will ensure a starch-free condition of the sapwood, though depletion may continue in certain circumstances after conversion; and thirdly, immersion in fresh water (an exceptional method of seasoning) serves to remove the starch, it being immaterial whether the timber is in log form or converted, so long as immersion follows very soon after felling; immersion subsequent to the death of the cells produces no change in starch content.

PART II.

STARCH IN RELATION TO *LYCTUS* ATTACK.

The importance of *Lyctus* powder-post beetles lies in the fact that they cause great damage to stocks of timber and to furniture, sports goods and many other commodities of which wood is a component. They confine their attention to certain "hardwood" species, the sapwood of which is reduced to a fine powder as the larvae eat their way through the wood. The damage may have proceeded some time before the presence of the larvae is apparent, because they do not perforate the surface of the wood, which may therefore appear to be sound when it is actually a mere shell. Ultimately the larvae become adult beetles which bore their way to the outside, leaving an exit hole which is sometimes the first indication of the damage already done. The life cycle recommences when the female lays her eggs below the surface of the sapwood.

Accounts of the species of *Lyctus* concerned, including their life history and habits, are given by Altson⁽¹⁾, and again by Fisher⁽⁶⁾, but these observers were unable to indicate the actual nature of the food of *Lyctus*. Fisher (*loc. cit.*), however, points out the importance of establishing what constitutes the food of the larva before further progress can be

made towards controlling the pest. Observations pointing conclusively to *starch* as the principal food of the *Lyctus* larva are here outlined.

The attack of a wood-eating beetle larva in a dry ash plank of unknown history was noticed to be definitely localised. The tunnels were limited to a well-defined region corresponding exactly to an area from which the bark had been missing from the time of felling the tree, as judged by the appearance of both bark and wood. The damage was further observed to extend to a depth of only 7 or 8 mm. below the exposed cambial face. Nowhere else in the plank was there any sign of "the worm." No living specimen was then present in the wood, but the nature of the tunnelling, and the quality of the dust or "frass" in the tunnels, indicated that *Lyctus* larvae had eaten the wood.

Microscopic examination by means of thin sections of both the infested and the uninfested sapwood revealed the fact that starch was present in abundance in the "worm-eaten" wood, but that it was absent from the rest of the sapwood. No other difference between the two was discernible.

This clue to the food of *Lyctus* was followed up by tests for starch among pieces of oak, ash and sweet chestnut timber from the same store as the ash plank just mentioned, and the occurrence of damage by beetles only in wood containing a considerable quantity of starch was confirmed. Sapwood devoid of starch, and that containing only a small quantity, were not infested although exposed to *Lyctus* attack equally with the others.

Since the *Lyctus* larva is known to eat its way through the wood, leaving behind in the tunnel which it bores large quantities of faecal matter in the form of fine wood dust (the "frass"), it seemed reasonable to suppose that the larva uses the starch as food. The next step therefore was to subject the digestive tract of the *Lyctus* larva to microscopical examination.

A *Lyctus* larva taken from ash sapwood was dissected under a binocular magnifier, and the whole gut was removed. The extensive crop was separated from the rest, and was placed on a glass slide in a drop of water. Pressure on the cover-slip served to spread the crop contents, which were then examined by means of a microscope. These were seen to comprise small groups of wood cells and parts of wood cells, and represented the wood which had been reduced to minute particles by the larval jaws and then ingested. The application of iodine solution made the numerous starch grains inside the cells visible. Starch was more prominent in the particles at the anterior end of the crop, less so posteriorly.

The contents of the rectum were treated in the same way, and while the wood particles were found to be identical with those in the crop as to structure, the cells were entirely devoid of starch. From this we conclude that starch is removed from the cells of the wood by the action of digestive juices, mainly in the intestine intervening between crop and rectum, but possibly also to some extent in the hinder region of the crop itself.

The "frass" from the tunnels in the wood was likewise treated with iodine and found to be starch-free except for a very few wood particles from which the starch had not been extracted. Hence it was very unlikely that these latter had passed through the gut. "Frass" can therefore be defined as consisting of the starch-depleted excreta of the larva plus an occasional particle of the original starch-bearing wood which the grub triturates with its jaws but does not eat.

The examination of the gut contents and of the "frass" therefore confirmed the supposition that starch was used as food by the *Lyctus* larva, and as no other source of food supply had become evident during the examination, there seemed to be ample proof that wood is only liable to infestation by *Lyctus* when it contains sufficient starch to nourish the larva. Mineral and nitrogenous substances remaining in the wood after the death of the cells doubtless form an essential part of the food of the larva, but it seems certain that the amount of starch present in sapwood determines its "nutritional value" for *Lyctus*.

A practicable method of controlling the *Lyctus* pest, therefore, would seem to be *the removal of the starch from wood by the log-seasoning process*. This supposition was put to proof. The experiment consisted in exposing to *Lyctus* attack both starch-free and starch-bearing samples of sapwood from the same log; *i.e.* samples which were as nearly as possible identical in all respects except for starch content. Suitable material was available as a result of the starch-depletion tests with wood from a young ash tree as described earlier in this paper. The test pieces were provided by the starch-depleted block which had had the ends sealed. This was split into sticks, and the ends containing vaseline were cut off so as to give the adult beetles every chance to lay their eggs in the starch-free wood. The controls consisted of the "rapidly air-dried," the steamed and the kiln-dried sticks taken from adjoining parts of the same tree, all containing the original amount of starch. All the test pieces and the controls were kept for a month at room temperature under the same conditions in order to equalise the moisture content, and were then placed at random in an insect cage. Numerous adult *Lyctus* beetles of both sexes were introduced in June, and the cage was then left undis-

turbed until the following February. The samples were then examined, and all the controls were found to be damaged and to contain full-grown larvae, but the starch-free sticks showed no sign of infestation. Proof had thus been obtained that starch depletion rendered ash sapwood immune from *Lyctus* attack.

LYCTUS AND AN "IMMUNITY STARCH LEVEL."

It frequently happens that among stocks of seasoned timber, such as dimension stock for furniture making, undamaged sapwood pieces are found among infested samples, and no satisfactory explanation of this fact has hitherto been forthcoming. The relationship between starch content and liability to *Lyctus* attack having now been established, sapwood remaining uninfested after exposure to attack would be expected to contain little or no starch. Tests for starch applied to numerous kinds and sizes of wood samples showed the correctness of that supposition, and when the history of the starch-free pieces was traced, it was found that they came from logs which had been depleted of starch by seasoning, since in ordinary sawmill practice logs are occasionally not converted till a year or more after felling, and as we now know the sapwood loses its starch in that time. Pieces from seasoned logs are often included in a consignment along with similar pieces from freshly felled timber, and hence the occurrence of *Lyctus*-free and *Lyctus*-infested pieces side by side.

Further investigations resulted in the finding of abundant starch in kiln-dried ash intended for aeroplane and motor-coach members, in steamed ash bends for sports goods, in oak furniture squares ready for machining, and in the sapwood of walnut intended for panels, after it had been steamed to make it dark in colour like heartwood¹. Although a process such as kiln drying "sterilizes" the wood (Stillwell (10)), so that any *Lyctus* larvae already in it will be killed, it does not alter the starch content. The discovery of the prevalence of so much *Lyctus*-liable timber served to emphasise the seriousness of the *Lyctus* problem, a subject which had already been referred to at some length by Fisher (6).

To determine the amount of starch that can be tolerated in a sample of sapwood which yet can be classed as *Lyctus* resisting is no easy matter. Obviously there must be a ratio of starch to wood substance which is just too low to nourish the larva, *i.e.* an *immunity starch level*. Much more information than is at present available would be necessary for the accurate gauging of the "immunity level"; an exact method of estimating

¹ Willow cricket bats and walnut gun stocks are dealt with in some detail in the sequel.

the starch content of wood is required, and samples graded for starch content would then be exposed to *Lyctus* attack, and the results noted. Such an investigation, however, is beyond the scope of this paper, though attention may here be directed to what appears to be an important subject for research.

Immediate diminution of the damage due to *Lyctus* need not await, however, the result of what might prove a protracted study. It has been shown that wood containing little or no starch is immune, and this condition can be detected by a careful observer after a little practice with the iodine test. If very little, or no, blue coloration (in minute spots or streaks) is seen when light-coloured sapwood is viewed through a lens of a magnification of, say, ten diameters, after the wood has been painted with an aqueous solution¹ of iodine and potassium iodide, the wood may safely be regarded as immune from *Lyctus*. Any greater quantity of starch should, for the present, be classed as unsafe. Dark-coloured sapwood calls for microscopic treatment, as, in this case, the identification of starch is more difficult.

LYCTUS IN WILLOW AND WALNUT.

Of the common timbers often infested by *Lyctus*, oak, sweet chestnut and ash of approximately more than forty years' growth agree in that each has a central, darker-coloured starch-free heartwood surrounded by a lighter-coloured sapwood, throughout which there is starch in winter. Willow and walnut, also commonly infested, differ from the foregoing species as to their starch distribution and present unusual aspects of the *Lyctus* problem.

Willow.

Willow is classified as a "fat" tree, *i.e.* one "containing little starch but much fat in winter" (Büsgen (2), p. 357). That *Lyctus* infests such a timber is unexpected and would suggest that an examination of willow might prove interesting. Such was found to be the case.

The end face of a winter-felled willow log presents a wide zone of white sapwood surrounding a light brown-coloured heartwood region. In a log of 18 in. diameter the sapwood may be 3 in. or so in width. The end face of such a log was made smooth, and iodine solution applied to the sapwood. No effect was detectable except at the outermost region, next to the bark, and here there was a narrow band coloured dark blue, indicating abundant starch (Fig. 9). Careful microscopic examination by

¹ Of sufficient strength to resemble port wine in colour.

means of transverse sections of the wood showed that the starch was definitely limited to the youngest annual ring of wood. All the rest of the sapwood contained fat but no starch.

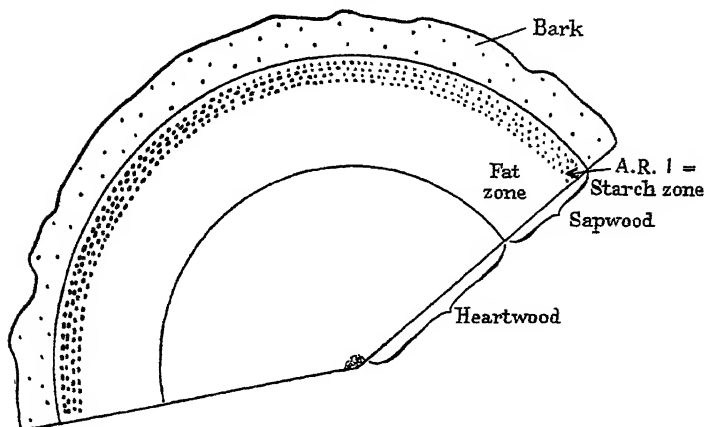


Fig. 9. Transverse section of willow tree felled in winter. Note starch in youngest annual ring only, A.R. 1.

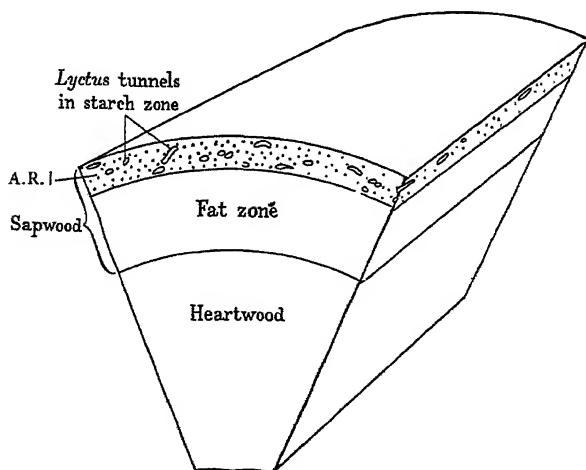


Fig. 10. Willow bat cleft (bark missing), seasoned ready for manufacture. Note starch zone with *Lyctus* tunnels, A.R. 1.

When this unusual feature came to light, enquiries were made to ascertain how far users of willow were troubled by powder-post beetles, and it was found that the stocks of willow at a cricket bat factory were heavily infested by *Lyctus brunneus*. An investigation into the incidence

of infestation produced some unexpected results, to understand which an account of the manufacture of bats will be necessary.

For cricket bats, selected willow trees (*Salix caerulea*) are cross-cut to length and the logs riven into segments by radial splits, thus producing "clefts" roughly triangular in cross-section (Fig. 10). The bark is stripped off, and the clefts stacked in an "open" fashion for rapid drying, otherwise the wood loses its white colour. Some time later the clefts reach the factory, and are there stored for a time to complete the seasoning process, this time in "close" stacks indoors. When ready for manufacture into bats some months or a year or more later, it is the rule to find the outer rim of each cleft reduced to powder by the "worm," or at an earlier stage of disintegration. In a well-grown willow the zone will be from $\frac{1}{4}$ to $\frac{1}{2}$ in. in (radial) width.

The damaged wood is, of course, removed when the bat is being shaped, and, moreover, the aim of the machinist is to remove all the "life" (as the outer zone is called), but it is difficult at this stage of manufacture to determine where one zone merges into another, as the boundaries of the annual rings are barely discernible. As a result, some shaped bats contain a portion of the last annual ring, and this is especially the case when a cleft is only just big enough to make a bat. Such a bat may show evidence of *Lyctus* infestation later in the process of manufacture, whereon it becomes necessary to scrap it, and it may even happen that in spite of the most careful inspection a bat may leave the factory while containing *Lyctus* larvae, and be returned later as "worm-eaten." Thus a good deal of loss is occasioned by the fact that the *Lyctus*-liable part of the sapwood cannot easily be determined, since, in the absence of visible damage, the amount to be removed has to be guessed or estimated in the light of experience.

When this problem was investigated in January, it was found that twelve months old willow clefts had abundant starch in the last annual ring, and none elsewhere, as had been expected from the custom of using winter-felled willow which had been dried quickly. Moreover, at that time, the *Lyctus* larvae were found only in the last annual ring. It seemed, therefore, that the only difficulty was to make sure of removing all the last annual ring during manufacture, and as the iodine test for starch was quite practicable in this case, the problem of *Lyctus* infestation in cricket bats appeared to be solved.

But it was pointed out that occasionally a "worm-hole" will appear in a finished bat, in a part of the wood other than the last annual ring (the annual rings being more easily identified on the finished surface).

As this seemed to indicate that the larvae penetrated into a starch-free zone, further investigation was necessary; so that clefts containing *Lyctus* larvae were kept, and from time to time examined and dissected by paring off shavings from the bark line inwards. In February many larval stages of *Lyctus* were found, and by using iodine solution on the wood as the paring proceeded, it was easily seen that the larvae were eating the wood of the youngest annual ring only, *i.e.* the starch zone, and skimming the boundary of the next annual ring, but not entering it. At that time no pupae were to be found.

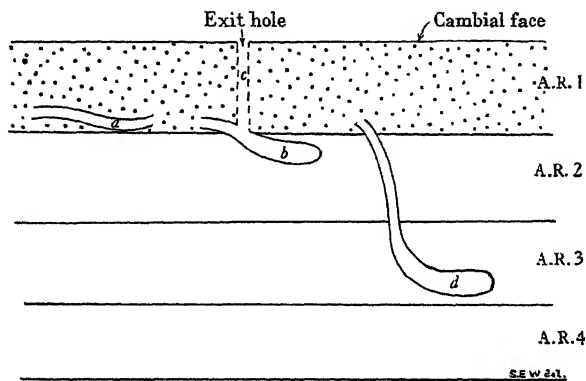


Fig. 11. Diagram of radial longitudinal section of cleft shown in Fig. 10, illustrating final stage of infestation by *Lyctus*. A.R. 1, youngest annual ring; A.R. 2, 3, 4, successively older rings. *a*, *Lyctus* tunnel skimming boundary of A.R. 2. *b*, pupal chamber within A.R. 2. *c*, exit tunnel of same. *d*, another pupal chamber deep in A.R. 3. The exit tunnel from this chamber would travel at right angles to the plane of the sketch to an outer surface nearer than the cambial face.

Later, however, in March, the infested clefts were examined in the same way, and a new and striking fact came to light. According to accounts of the usual habits of *Lyctus*, the full-grown larva generally makes a chamber near the surface of the wood, there pupates, and has only a thin surface layer of wood to penetrate before it can emerge as an adult beetle. In these willow clefts, however, it was found that the full-grown larva left the starch zone, and, tunnelling *inwards*, excavated a pupal chamber in the "fat" zone of the sapwood. Most of these pupal chambers were found in the second latest annual ring, but in a few cases the larva had penetrated even deeper, so that pupae were found at a distance of $\frac{3}{4}$ in. from the starch zone (Fig. 11).

By June the adults had all emerged, leaving exit holes. When the exit tunnels were traced from the holes inwards it was seen that the

beetles had taken the shortest line from the pupal chamber to the outside, so that while most of them had emerged on the surface of the first annual ring, some of the exit holes appeared on the radial faces of the clefts.

Therefore an explanation of the solitary "worm-hole" which may appear in a finished bat seems to be that the bat is shaped after the larvae have become pupae. Now although most of the pupae will be removed during the discarding of all wood in which the damage is obvious, yet a "frass"-packed tunnel in the deeper wood may be missed, and consequently a pupa may be left undisturbed while the bat is being finished. The adult beetle later eats its way out to the nearest face, leaving a solitary exit hole.

It was interesting to find, from microchemical tests of the gut contents of *Lyctus* larvae found in willow, that a "feeding" grub has starch in the crop and none in the rectum, but that a deep-tunnelling full-grown larva has no starch in its gut at all, but fat is present in the wood cells of both crop and rectum. Thus although such a grub eats the wood of the second annual ring, it derives no nourishment therefrom. Indeed, it is obvious that *Lyctus* can make no use of the plentiful reserve *fat* available in the sapwood of willow.

With regard to the production of cricket bats it is suggested that manufacturers should employ the iodine test for detecting starch in the clefts as they arrive at the factory, and in this way ensure the removal from the cleft of all wood capable of feeding *Lyctus* larvae. It must be remembered, however, that unless the last annual ring is removed within about six months of possible infestation, whether the exposure to attack was prior to arrival at the factory or not, there is always the risk that the larvae will be ready at that time for pupating, and will have penetrated the second latest, or even older, annual rings, with a consequent increase in the amount of damaged wood to be discarded, and the possibility of a faulty bat at the finish.

In the case of logs which would provide clefts too small to make bats without utilising the last annual ring, the cleaving should be delayed and the bark kept on for a few weeks until the starch in the last annual ring has been depleted. Since the starch in the sapwood of willow is limited to a narrow band, and this band is next to the bark, depletion would be much quicker than in the case of oak, for example, so that probably there would be no considerable loss of whiteness due to seasoning willow in the log. In any case, perhaps, the present fashion for white cricket bats will pass, and bats with more colour be preferred.

Walnut.

While the sapwood of walnut agrees closely with that of oak as to starch content, the *heartwood* of walnut exhibits an unusual feature in containing considerable quantities of starch. The starch is present in all the heartwood, chiefly in the outer part of each annual ring (the "late" wood), but varies in amount from ring to ring¹.

When this fact came to light the effect of log seasoning was tried. A walnut tree was felled in January, and a log, with the bark intact, was kept and examined at intervals. The sapwood was about 3 in. wide, and in eighteen months the sapwood starch, at first abundant, had all been cleared, but *the heartwood starch remained unchanged*.

Now walnut is well known to be extremely liable to damage by wood-boring beetles, and the question arose as to whether the presence of starch in the heartwood had any bearing on this fact. An inspection of a number of walnut scantlings at a Government rifle factory where *Lyctus* was known to cause great loss (see Fisher⁽⁶⁾, p. 11) served to show, however, that in no instance was *Lyctus* infestation found in the heartwood. As there appeared to be sufficient starch in the heartwood to nourish *Lyctus*, the conclusion must be that the heartwood is distasteful to the larva, possibly by reason of the presence of other cell contents and cell-wall impregnations.

Walnut sapwood is normally quite light in colour and the starch is therefore easily detected, but the stocks at the factory concerned had all been steamed before arrival so as to render the sapwood dark in colour like the heartwood. Starch was not easily seen in consequence, but could be detected by careful examination with a lens after the usual iodine treatment. It was thus quite possible to distinguish the few starch-free scantlings among the many starch-containing and *Lyctus*-infested pieces. The marked susceptibility of walnut timber to *Lyctus* infestation must be related, therefore, not to the occurrence of starch in the heartwood, but to the large proportion of starch-bearing sapwood, which is apparently "permitted" in specifications for rifle stocks (and furniture), when it has been rendered similar in appearance to heartwood by a process of steaming.

The fact that starch is cleared from the sapwood of a walnut log by seasoning "in the round" is in keeping with all previous experiments, and it can be stated with confidence that the heavy expense of constantly

¹ The occurrence of starch in heartwood is surprising since such starch can never be used by the tree.

sorting the stocks of walnut and the wastage of much very expensive timber can be saved by the simple expedient of requiring all supplies (which come entirely from abroad) to be seasoned in the log sufficiently long before conversion to clear all the starch from the sapwood. Each piece would then be tested on arrival at the factory, and all which contained starch would be rejected as not being in accordance with the specification. The heartwood starch, as has been shown, can be disregarded. Many hundreds of pounds a year can thus be saved to the national exchequer in the one factory alone.

STARCH CONTENT OF WOOD IN RELATION TO FUNGAL DECAY.

An examination of ash planks which had been seasoned in the open air suggested that a relationship existed between the starch content of wood and its liability to fungal attack. The faces of planks known to have been cut shortly after felling were seen to be badly discoloured, while planks from logs converted six months or more after felling remained "bright." Microscopical examination of the discoloured wood disclosed the presence of brown fungal hyphae in the wood to a depth corresponding to the starch zone previously noticed in ash plank (p. 673). Further, when a pile of discoloured planks was disturbed, the position of the "stickers," or separating strips, was clearly marked by a bright clean stripe across the plank. Here there was neither starch nor fungus. All the facts pointed to the presence of the fungus being determined by the occurrence of starch at the discoloured surface, for where the "stickers" protected the surface from drying quickly, the starch disappeared for the reasons already stated.

In order to test the hypothesis that the presence of starch as food for fungi determined the liability to fungal invasion, matched samples of starch-containing ash sapwood, "rapidly air-dried," steamed, and oven-dried, along with a matched sample of starch-free ash (*i.e.* part of the same material as served for the *Lyctus* infestation test, p. 678), were placed in a culture of a fungus (unidentified) known to cause "sap stain" in ash. After five weeks the blocks were split, and from the obvious discoloration the fungal invasion could easily be traced. Whereas the first three blocks were stained throughout¹, the starch-free samples had only a slight growth on the surface and no internal discoloration.

Portions of the same blocks were likewise placed in culture tubes after wetting and exposure to chance infection, with significant results. The

¹ A small area in one block not affected was found on microscopic examination to be starch-free.

starch-free block remained mould-free a week longer than the rest, and had only a slight and superficial growth after five weeks, while all the starch-bearing blocks had a very profuse growth.

These results show that in ash the starch content is a measure of the nutrient value of the wood for common moulds and sap-staining fungi, *i.e.* organisms that feed on the cell contents.

DISCUSSION AND CONCLUSION.

Previous investigators have aimed at tracing a relationship between the "age" of timber and its liability to attack by *Lyctus*, and between the moisture content of the wood and the rate of growth of the larva (Fisher⁽⁶⁾). Failure in these attempts is probably to be explained by the ability of the powder-post beetles to tolerate wide variations in the physical conditions of their natural habitat (dead trees). Certain species of *Lyctus* are stated to oviposit only in the exposed ends of the wood vessels, the "pores," and not in cracks or fissures. If that be so, then some importance attaches to the dimensional relation between the pore and the ovipositor and egg of the beetle. From a careful series of measurements Clarke⁽⁴⁾, p. 31 arrived at the conclusion that "pore size must be regarded as a limiting factor in the attacks of *Lyctus*."

Apart from any mechanical obstacle to egg-laying, we now know that the criterion of infestation is the occurrence of starch in sufficient quantity to nourish the larvae; the "feeding-marks" described by Fisher (*loc. cit.* p. 3) are probably to be correctly interpreted as tests, by the adult female beetle only, of the starch content of the wood in question, prior to egg-laying.

So long as the actual food of *Lyctus* remained unknown, it was obviously impossible to make any real progress towards a solution of the problem of infestation. Concentration on the physical aspects of the subject and neglect of the biological factors seem to explain why the larval food has not been discovered earlier. This is all the more surprising when it is seen that there have frequently been clear indications of the importance of cell contents in accounts of earlier investigations. Thus Snyder⁽⁹⁾ observed that the submergence of "hardwood" logs in water for four months rendered the wood immune from *Lyctus* attack, and concluded that a change took place in the food value of the cells. Then Fisher⁽⁶⁾ thought it possible that changes in the chemical composition of the cells, occurring during seasoning, might have some bearing on the problem. Fisher (*loc. cit.*) noticed that the rate of development of the larvae varied considerably, and ascribed this to "differences in the

nutritional value of the various samples of wood." From a chemical analysis of *Lyctus* "frass," and comparison with "normal" oak sapwood, Campbell (3) concluded that "the source of larval nourishment is within the cells and not in the cell wall substance," though the occurrence of starch in his "normal" sapwood was not observed. As long ago as 1903, Mer (7) suggested that there was a relationship between "*vermoulure*" and the amount of starch present in the wood, though it should be pointed out that Mer's experiments were with living trees and not with felled timber.

The treatment of felled timber in the manner advocated in this paper, viz. log seasoning, is here offered as a solution of the problem of *Lyctus* infestation, as it is a simple and inexpensive method of rendering timber immune from attack. No costly impregnations with insecticides are necessary, and very little extra handling need be involved. Such log seasoning already takes place to a relatively small extent for quite other reasons, e.g. sawmill convenience, so that immunity from *Lyctus* is already a feature of a certain proportion of English timber stocks. Wherever powder-post beetles are prevalent, it is necessary to seal the ends of the logs with paint or limewash to prevent egg-laying by the adult beetles, otherwise the larvae will damage the wood while starch depletion is proceeding.

The discovery of the minimum time required for seasoning different species of timber "in the round" so as to render the wood immune from *Lyctus* infestation is of importance commercially, and it is suggested that the compiling of a time schedule might be undertaken, following the determination of the "immunity starch level" for *Lyctus*. Further study of the anatomy and physiology of the *Lyctus* larva, particularly as to its digestive system, might throw valuable light on the physiology of wood-eating insects in general. In this paper the bearing of the starch content of wood on the occurrence of sap-staining fungi has been indicated; this line of investigation might usefully be extended to include fungi causing decay.

The results obtained from the study of *starch* in timber during the course of the present investigation point to the necessity for a new direction in research work on the relationship between invading organisms and the wood they damage, since the importance of the contents of the xylem parenchyma can no longer be ignored.

SUMMARY.

1. The starch present at the time of felling in the xylem parenchyma of the sapwood of timber trees is shown to disappear when drying is delayed, as in the case of timber kept in log form for a period of time varying with the species of tree, the season of felling, the width of the sapwood, and temperature.

2. It is suggested that starch depletion results from the continued activity of the sapwood cells. This view is supported by experimental evidence of the respiration of wood.

3. Starch depletion by log seasoning is a method of preventing the infestation of timber by *Lyctus* powder-post beetles since starch is proved to be the main food of *Lyctus* larvae.

4. When timber is "converted" soon after felling and then subjected to treatment (e.g. kiln drying or steaming) which kills the cells, the starch remains in the sapwood. Such timber remains liable to *Lyctus* attack since the starch content is not altered by any subsequent (seasoning) treatment.

5. The removal of starch renders sapwood resistant to sap-staining fungi.

ACKNOWLEDGMENTS.

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I also record my gratitude to Prof. V. H. Blackman for guidance in the preparation of this paper.

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Extensive bibliographies will be found in BÜSGEN (2) and FISHER (6).

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GNARLED STEM CANKER OF TEA CAUSED BY THE CAPSID BUG (*HELOPELTIS BERGROTHI* REUT.)

By R. LEACH, B.A., A.I.C.T.A., *Mycologist*

AND

COLIN SMEE, D.I.C., F.E.S., *Entomologist*.

(*Department of Agriculture, Nyasaland.*)

(With Plates XXXI and XXXII.)

INTRODUCTION.

FOR many years a stem canker of tea has been known in Nyasaland. Several reports of the disease have been made, but until now the cause of the disease has been associated with fungi. This has also been the view of workers in other tea-growing countries.

This paper describes our work on this disease during 1932. We consider that we have collected sufficient evidence to show conclusively that the disease is caused by the feeding of the tea mosquito bug (*Helopeltis bergrothi* Reut.) on the more mature green stems of tea. We consider that fungi play a purely secondary part in the etiology of the disease.

We suggest the name "gnarled stem canker" as being a more descriptive name for the disease than "stem canker" or "branch canker," which may include many different diseases.

REVIEW OF LITERATURE.

The term "canker" has been used to describe a number of diseased conditions occurring on different parts of the tea bush and which have been attributed to a variety of causes, mainly fungi. The indiscriminate use of the terms "stem canker" and "branch canker" leaves one in doubt as to the actual part of the bush attacked, and the matter is still further obscured by the fact that in many cases of canker, isolation of a causal organism has not been successful.

Petch (13) classifies branch canker into three types: "(1) wounds or gnarled formations on young branches (red wood); (2) open wounds, often extending for a foot or more, along the upper surface of old horizontal

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branches; and (3) wounds on old vertical or horizontal branches which extend along the branch from an old pruning cut."

Gadd (5) has suggested that the name "wood rot" should be applied to that form of canker which is characterised by the presence of rotted wood, and presumably this term is mainly applicable to Petch's second and third types. It appears that the gnarled type of wound is to be found under the varying conditions of tea cultivation in both India and Ceylon, and the diversity in the appearance of such stems, as noted by Petch (13), is accounted for by the fact that somewhat similar results are produced by more than one cause.

In Ceylon, a canker that is more of a wound than a gnarled type is definitely attributed to the fungus *Macrophoma theicola*. The original report from India of canker is to be found in Watt and Mann (18), and it is there stated that the cause was *Nectria (ditissima?)*. These authors, however, point out that "in every case there must have been previous injury by insects, by pruning, by lightning, or by other agents. It (the fungus) appears not to be capable of attacking a healthy uncut tea stem."

The first report of the gnarled canker condition in Nyasaland was made in 1926 and it was associated with a *Macrophoma* fungus. The appearance of the attacked stems, however, was not entirely the same as that definitely attributed to *M. theicola* in Ceylon. In 1927 Butler (1), working on the disease in Nyasaland, came to the conclusion that there was a connection between a fungus mycelium found in the diseased areas and the cankerous condition, but he also states (1): "the cankers are less regular in appearance than those caused in Ceylon by *M. theicola*, the callus ring surrounding them is less uniform and less prominent, and they are often partially covered by shreds of bark. They are, however, exceedingly like one of the forms of branch canker known on tea in India without any clue having, as yet, been found as to its cause."

In 1928 Tunstall (16) reported from Assam as follows: "Another disease of stems which does not appear to be directly due to vegetable parasites is the condition known as canker. The young wood swells irregularly, thus presenting a cankerous appearance. This condition is frequently associated with severe attacks by insects such as the 'mosquito bug.'" In the *Tea Quarterly*, vol. III, August, 1930, the following statement was made (11): "... The other type of branch canker was received from South India where it is considered to be a serious disease. So far it has not been reported in Ceylon. The cause is possibly of mycological origin but attempts to isolate a causative organism have not proved successful."

It is evident that the canker found in Nyasaland is similar to, if not identical with, that reported from India, and the illustration in Watt and Mann (18) represents the typical final condition of the cankers as we know them (Plate XXXI, fig. 3).

In 1930, specimens that were considered to be the earliest stage of the disease were sent from Nyasaland to the Imperial Mycological Institute. After examination of this material Dr Butler reported: "There is considerable similarity between the early stages of tea canker and that of the cankers caused by the common woolly aphis (*Eriosoma lanigerum*), the anatomy of which has been carefully worked out." He considered that "the anatomical condition suggests first an insect cause and secondly a localised bacterial trouble." It was these statements that finally led us to consider the possibility of mosquito bug being connected with the disease.

PREVIOUS OBSERVATIONS AND EXPERIMENTS IN NYASALAND.

It was noticed that cankers appeared chiefly where young tea had been grown in badly ventilated areas such as nurseries surrounded by high grass, or in gardens where pigeon pea (*Cajanus indicus*) had been allowed to grow to a considerable size. When first noticed the disease "was worst in hollows" where the soil "is moist and badly drained" (1). Lately it has been associated regularly with pockets of black waterlogged soil in some districts.

Control measures in the past aimed at alleviating the above conditions, together with cutting back diseased stems below the cankers with the object of preventing the supposed fungus organism from passing down into the root and thereby killing the plant. Later observations showed, however, that after 9 months the cankers did not increase in size and no fungus passed down the stem. Inoculation experiments with various fungi isolated from cankered stems gave negative results.

Sometimes new branches were noticed growing out from the cankered areas. Cankers were usually first noticed at the end of the rains and the effects continued to appear through the dry season. The affected stems often died back in the dry season but this die-back only progressed as far as the lowest limit of cankered areas. In the following year healthy branches grew out below the cankers.

Plants grown from imported seed seemed to be more susceptible to attack than the local jat plants, although the latter were occasionally found to be badly attacked.

TECHNIQUE.

(i) *Microscopic examination of cankers.* Specimens were fixed in formalin-acetic-alcohol and cut with a Spencer hand microtome. The best double stain was found to be safranin and Delafield's haematoxylin.

(ii) *Bagging experiments with mosquito bugs.* These were carried out by means of mosquito net "sleeves" tied on to the tea stems. The leaves were cut off close to the stem inside the sleeved portion, but the leaves at the top of the stem were left so that normal growth would be interfered with as little as possible.

(iii) *Examination of stylets in situ.* A method similar to Smith's⁽¹⁵⁾ was employed. Hot wax was dropped on to the heads of the feeding insects, and the stylets were severed from the heads before putting the stem with insect attached into xylol. This precaution prevented the weight of the insect's body displacing the stylets when the wax was dissolved. The stem and stylets were then put into an absolute alcohol-xylol solution and afterwards into absolute alcohol.

Serial transverse sections were cut with the hand microtome. The stylets could be seen clearly in the tissues with a hand lens. Microscopic examination was made, however, in order to determine the exact position of the ends of the mandibles and maxillae.

(iv) *Measurement of cankers.* These were made in the laboratory; the cortex was removed and only well-defined cankers were measured as some were formed too close to each other to be distinguished individually.

CAUSE OF THE DISEASE.

Four insects were bagged singly on each of the following types of green stem: (a) next to pencil wood; (b) near the terminal bud; and (c) intermediate between (a) and (b). As controls, four stems were bagged without insects. The stems were bagged for 24 hours.

Typical first stages of canker (Plate XXXI, figs. 1, 2) were formed in (a) and (c); no typical canker was found in (b) but only lesions typical of those found in young flushes of tea on which the insect usually feeds. The controls remained healthy. After 2 months the cankers gave the stems the gnarled appearance which is so characteristic of the later stages of the disease (Plate XXXI, fig. 3).

This experiment showed that the first stage of gnarled stem canker of tea had been produced by mosquito bugs feeding on the green stems.

Later experiments showed that, except for the smallest nymphs, mosquito bugs of all ages are capable of forming cankers. Adults and the

larger nymphs can feed on pencil wood when compelled, in which case cankers are also formed.

Other experiments were carried out to investigate:

- (i) the morbid anatomy of the early stages of cankers;
- (ii) the size of cankers caused by insects of varying ages.

During the course of these experiments it was found that a small percentage of bagged insects did not form cankers. This anomalous behaviour may be explained by the following reasons: nymphs do not feed for 24 hours before casting their skins; insects may be deterred from feeding by being in a strange environment; deaths sometimes occur.

DESCRIPTION OF INSECT AND ITS FEEDING HABITS.

The insect, *H. bergrothi* Reut., called herein the tea mosquito bug, is a typical capsid bug and is widely distributed in Africa. Its life history and bionomics do not disclose any noteworthy departure from the normal. It is known as a pest of some importance on cocoa in the Gold Coast⁽¹²⁾, on cotton in Nigeria⁽⁶⁾, and in the Belgian Congo⁽¹⁰⁾, and has been recorded from a wide variety of plants in East and Central Africa. As a pest of tea in Nyasaland it has never so seriously affected the flush as the species of *Helopeltis* in India is reported to do, but its presence in the tea gardens has been continually noticed for several years.

Its life history and the damage it causes to the tea flush, under local conditions, are described elsewhere⁽¹⁴⁾. The insect's proboscis is normal in structure, consisting of a pair of apically barbed mandibles and a pair of needle-like maxillae moving in a jointed rostrum. Feeding takes place during the night and early morning, but during bright sunlight the insects take refuge on the lower surface of leaves in the inner part of the tea bush. The wingless nymphs move up and down the stems with considerable activity. The winged adults when disturbed, either sidle rapidly to the lower surface of a leaf or take flight for a short distance and then drop into another bush. The very smallest nymphs are most usually to be found on the small leaves at the base of or towards the centre of the bush. These habits are of some interest, as it appears from them that the older nymphs are likely to pass a greater length of time on the green shoots than either the adults or the small nymphs. Oviposition, however, brings the adult females into contact with such shoots and feeding on them may then be expected to, and certainly does, take place.

The length of time an insect will remain feeding in one position is very variable. The longest period observed was 20 min. in the case of an adult

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feeding on a green stem. But this period of time has little bearing on the size of the canker produced. A point that has been found important, however, is that an insect may feed in several closely adjacent spots, and as each punctured area may give rise to a canker, the stem may be completely "girdled."

In the field, mosquito bug has been observed to be most prevalent in areas having certain ecological conditions, such as poorly drained sections of a tea garden or nurseries thickly enclosed by jungle grass, and it is worthy of note that the canker disease is recorded as being most prominent in similar places. This matter is referred to later in the section on control measures.

DEVELOPMENT OF CANKERS.

(1) *Macroscopic.* Sometimes even before the insect stops feeding a faint water-soaked area is seen round the spot where the stylets entered. This area becomes more definite within an hour. The density of the chloroplasts in the cortical cells regulates the clarity of the canker, which is therefore seen clearly by scraping off the cortex (Plate XXXI, figs. 1, 2). The canker is then seen as a light brown, oblong ovate discoloration. At first the canker is darker at the circumference than at the centre. After 24 hours the colour becomes a uniform dark purple-brown. These stages have not been previously described.

The canker remains unchanged while the cortex is living. There is no sign of the canker when cork bark first grows over it, but in due course a small sunken area marks the position of the canker. This is an indication of gall wood commencing to grow round the edge of the canker. The development of gall wood round each canker gives the stem a gnarled appearance owing to the short distances between individual cankers (Plate XXXI, fig. 3). The original discoloured area of individual cankers may be found by cutting away the gall wood.

The later stages of canker development may show blackening of the surface, which indicates the entry of saprophytic or weakly parasitic fungi. The original cankers may be found separated from each other by healthy tissues when the gall wood is removed, but in most cases the proximity of the cankers enables the fungi to travel from one canker to another and in consequence the individuality of the original cankers becomes indistinct.

Cankers vary considerably in their length and breadth. Table I gives measurements of cankers on green stems of tea growing in an Indian jat nursery.

Table I.

Measurement of natural cankers.

	No. of plants	No. of cankers	Mean (mm.)	Standard deviation	Standard error of mean
Length	21	176	16.835	± 3.872	0.2917
Breadth	21	176	1.906	± 0.709	0.0534

(2) *Microscopic.* Usually feeding by the tea mosquito bug takes place on the youngest leaves and the very young stems. The cortex is discoloured in the latter case. The cells which collapse are situated outside the phloem and the pericycle (Plate XXXI, fig. 5) and do not interfere with their development. The pericycle ring later becomes lignified so that the collapse cells are separated from the vascular system (Plate XXXI, fig. 6). The surface of the stem becomes "russeted" in the affected area (Plate XXXI, fig. 4).

If, on the other hand, the attack is on older green stems in which the pericycle fibre ring is lignified, a canker is formed when the insect feeds in the pericycle parenchyma or phloem. Feeding in the xylem results only in a faint discoloration of the xylem parenchyma and medullary rays; feeding in the cortex results in premature bark formation.

For approximately 1 hour after the insertion of the stylets (Plate XXXII, fig. 7) the parenchymatous cells of the pericycle remain normal in shape but their *walls* become slightly brown. They then start to collapse tangentially. Plate XXXII, fig. 8, shows the first signs of the collapse, which progresses rapidly, and within a few hours the collapse of the whole pericycle parenchyma is complete (Plate XXXII, fig. 9). Within 24 hours the phloem has also collapsed (Plate XXXII, fig. 10). The collapse of these tissues is the most interesting and important feature of the anatomical aspect of the disease.

The development of the canker from this point onwards is connected with the growth of gall wood. It is interesting to note that when plant growth is slow the cortex remains healthy while gall wood continues to be formed on the inside of the necrotic area (Plate XXXII, fig. 11). Some cankers have been noticed to remain in this condition for 2 months in the cold season. Gall wood takes the place of the normal phloem and xylem development, and continues to grow and keep pace with the development of the healthy xylem until the bark forms over the necrotic area. Dead cells are then situated on both sides of the pericycle fibre ring.

At this point in the development of the canker the cambium may or may not be killed. In the first case growth is checked below the necrotic

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area and gall wood continues at the edge of the canker, forming a callus lip (Plate XXXII, fig. 12). In the second case gall-wood growth is unchecked.

As the description of the morbid anatomy of the cankers from this stage has been given by Butler (1,2), we have not made a further detailed examination of the canker beyond this point. Butler (2) states: "The new growth is due to cambial stimulation on the xylem side, as in the woolly aphid gall. The gall-wood elements are often not in radial continuation with the old, but are variously bent or contorted, sometimes even coming to lie across the rows. The pressure ruptures the cortex and the bark becomes rapidly exfoliated into shreds. Sometimes the cambium is killed over a part of the circumference and this leads to a complication of the structure by attempts at a normal process of wound repair extending in from the cambium at the sides. The wound may be entirely closed, or repair may fail to close it and a gaping canker down to the original wood remains, bounded by swollen callus lips."

Fungi and bacteria have not been found associated with a canker until the cortex collapses. Various fungi soon become evident then on the surface and hyphae have been found penetrating the necrotic tissue. The weakening of the stem due to the ringing effect of the cankers probably accounts for the penetration of the neighbouring tissues mentioned by Butler.

PATH OF STYLETS IN THE STEM.

Experiments carried out by killing insects while feeding on the stems gave the results shown in Table II.

Table II.

Position of stylets in stem in relation to canker formation.

Number of insects fixed *in situ* = 40.

	Stylet points found in				
	Cortex	Pericycle parenchyma	Phloem	Xylem	Pith
Cankers present	0	18	1	1	0
No cankers	6	1	0	13	0

As expected, the tips of the stylets were only found in pericycle parenchyma when a canker was formed. The stylets are also found, however, to end in the xylem, cortex and pith. Experience showed that the original method of starving the insects prior to feeding on stems favoured one or other of these latter positions. If the insects were allowed to feed regularly cankers were formed more frequently. Feeding in the wood was,

therefore, probably induced by the hungry insects pushing in their stylets farther than usual in their anxiety to feed.

When the stylets ended in the xylem and a canker formed, it might be supposed that the insect moved its stylets inwards after feeding for some time in the pericycle parenchyma. This supposition is upheld by observation of the insect while feeding. Occasionally after feeding in one position the insect, without withdrawing its stylets, is seen to push its head close to the stem as if pushing its stylets deeper into the tissues.

The stylets may twist considerably after entering the stem. They penetrate through the pericycle fibre cells or through the thin-walled cells of the gaps between. In the majority of cases where cankers are formed the stylets have been found to pass through the gaps in the fibre ring.

The stylets pierce the tissues intercellularly. When fixed *in situ* no saliva is apparent close to them. On the other hand, the stylet track is often marked by a brown discoloration in the cortex *after* they have been withdrawn. The cells close to this track in the cortex stain deeply with safranin (Plate XXXII, fig. 10). The question of saliva injection is discussed later (p. 702).

SIZE OF CANKERS.

Experiments were carried out to study the extent of damage which can be caused by the different life stages of the insect. In this way it was hoped that some indication might be obtained with regard to which stage was most probably concerned with the damage caused under natural conditions.

We are indebted to Dr J. Wishart, School of Agriculture, University of Cambridge, for kindly assisting us in making a critical examination of our experimental data. Unfortunately, by comparison of the measurements of cankers produced under natural and experimental conditions, we have been unable to draw any conclusion as to which stage of the insect is most closely concerned with the cause of the disease in the field.

Exp. I. Ten adult male insects were bagged on different plants and were allowed to feed for 24 hours. Each insect was numbered. The sleeves were the same size and each was tied just above the pencil wood. They were then removed and the procedure was repeated on the two following days, fresh plants being used each day.

Although cankers caused by the same insect may vary considerably on different plants, the differences in the mean size of cankers produced by different insects of the same age approach a significant level (P just under

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0.05). Thus, if there is a difference, it is not very large and it may not have been demonstrated beyond all doubt.

Table III.

Difference between size of cankers produced by similar insects on stems of similar ages.

Analysis of variance.												
Due to	Degrees of freedom	Sum of squares	Mean square									
Days	2	15.60	7.80									
Insects	9	93.30	10.36									
Error	18	75.77	4.209									
Total	29	184.67										
$Z=0.4504$, 5 % Pt =about 0.45.												
Summary.												
	Number of insect										Mean	Standard error
	1	2	3	4	5	6	7	8	9	10		
Mean length (mm.)	13.9	17.97	18.5	17.33	18.8	15.07	14.7	16.6	15.0	17.0	16.46	1.185

Exp. II. The results shown in Table III might well be due to some varying plant quality or to the stems being of different ages. An experiment was therefore carried out to test whether stems of different ages produced cankers of different sizes. Two insects were sleeved on to one plant, one below the youngest (most supple) part of the stem and the other just above the pencil wood. Ten plants were bagged in this way.

Table IV shows that the significant difference between the size of the cankers produced on the two different aged stems is very marked—the larger cankers being formed on the younger portions of the stem.

Table IV.

Difference between the size of cankers produced by similar insects on stems of different ages.

Analysis of variance.				
Due to	Degrees of freedom	Sum of squares	Mean square	
Plants	9	57.2	6.356	
Insects	1	57.8	57.8	
Error	9	37.2	4.133	
Total	19	152.2		
$Z=1.3190$, 1 % $Pt=1.1786$.				
Summary.				
	Older green stem	Younger green stem	Mean	Standard error
Mean length in mm.	14.1	17.5	15.8	0.643

Exp. III. An experiment was carried out to test the possible difference in size of cankers caused by the four older stages of the insect. The numbering of the nymphs indicates the number of times they had cast their skins. Except for adults and the largest nymphs, which cause cankers of much the same size, it is seen from Table V that there is a significant difference in the size of cankers caused by the various stages of the insect.

These results are interesting and show the varying size of cankers produced by the various sizes of insect. These measurements should be compared with the figures for natural cankers (Table I), namely 16.835 and 1.906, which are not far removed from the weighted mean of the cankers caused experimentally (below). From these results, therefore, it is impossible to assign responsibility for the greatest amount of damage in the field to any one stage of the insect.

Table V.
Size of cankers caused by different stages of insect.

	No. of insects	Mean	Standard deviation	Difference between means	Standard error of difference between means
Length (mm.)					
Adult	149	18.92	± 3.406	0.37	± 0.4576
4th nymph	140	18.55	± 4.292		± 0.4661
3rd "	147	15.01	± 3.549		± 0.3963
2nd "	57	11.07	± 2.016		

Weighted mean length = 16.74 mm.

Breadth (mm.)					
Adult	149	1.88	± 0.5545	0.05	± 0.059
4th nymph	140	1.93	± 0.4533		± 0.049
3rd "	147	1.52	± 0.3693		± 0.056
2nd "	57	1.17	± 0.3515		

Weighted mean breadth = 1.70 mm.

These experiments show the great diversity in the size of individual cankers. It can be understood readily how a few cankers formed close to each other can ring stems averaging about 15 mm. in circumference. It was shown that this could be done in one night by a single insect.

DISCUSSION.

The explanation of the cause and development of gnarled stem canker of tea in Nyasaland is, we consider, clearly accounted for by our results. Control measures can now be based on definite lines whereas in the past they have only been of a tentative nature. It is clear that the organism to be controlled is not a fungus, it is the tea mosquito bug.

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Up to now the depredations of this insect have been considered to be confined mainly to the young flush. It is interesting to know that its activities can cause this sometimes serious disease of stems of the tea plant.

There appear to be few records of capsid bugs producing malformations of plant stems in a similar manner to that now described, though damage to fruits and foliage is frequently reported. Light (9) refers to the fact that *Helopeltis* in Ceylon feeds to some extent on the stems of the tea bush, but no development of a cankerous condition appears to be recorded as the result. Golding (6) refers to *Helopeltis* attacking cotton in Southern Nigeria and notes that the terminal shoot may die as a result of damage to the main stem. Patterson (12) describes the same insect as feeding on the soft stems of cocoa as well as the pods and young leaves. In neither case, however, does it appear that any specific diseased condition of the stems, developing later, has been associated with such feeding. On the other hand the latter author (12) describes a condition on the stems of cocoa, which though not strictly comparable to our tea canker appears to be in the same category, caused by the feeding of two species of *Sahlbergella*, and this condition is also described and figured by Cotterell (3). More recently workers in America have shown that various plant-sucking insects, in particular the cotton flea hopper (*Psallus seriatus* Reut.), are capable of causing lesions on the stems of cotton plants (4, 7). Van Hall (17) describes the damage done to cocoa stems by a species of *Helopeltis* in Java. He figures the effect on the young stems. The lesions may be compared to some extent with those shown in figs. 1 and 2 of our paper. But here again the resulting damage does not seem to be identical with our tea canker.

The most interesting part of the disease is connected with the collapse of the tissues in the first 24 hours after sucking by the insect and the stimulus which causes this collapse. From our investigations we consider that this stimulus cannot be due to mechanical injury owing to the fact that it is felt at a considerable distance (up to 12 mm.) from the point where laceration of the cells occurs. The collapse seems too rapid for fungi or bacteria to be concerned; neither type of organism has been isolated from the earliest stage of the disease, nor have they been seen in the collapsing cells.

We are therefore left with the alternative that the stimulus causing the initial collapse is due to the injection by the insect of some irritant that diffuses through the cells from which sap is sucked. It has been observed that no exudation of saliva takes place through the proboscis on

to the plant surface immediately prior to sucking, but occasionally a drop of fluid appears in the centre of the punctured area after withdrawal of the stylets and this is more particularly noticeable when a leaf is sucked.

The nature of the irritant is of interest but we are unable at present to state what form this takes. The subject of the toxic effect on the tea bush and other plants of the feeding of capsid bugs has received considerable attention from a number of workers but reference to the literature leaves one in doubt, in many cases, as to whether the exact nature of the irritant is (a) salivary injection, (b) enzymic, or (c) chemical.

Smith⁽¹⁵⁾ states: "In examining capsid bug punctures in the tissues of the potato leaf, the cells lying in the injured area are found to be blocked up with a granular substance which, like the stylet tracks of the aphid, very readily picks up safranin stain. The question arises as to what is the nature of this substance. Is it the salivary secretion of the insect alone or is it formed by a reaction on the part of the plant to the secretion? It seems probable that it is a combination of saliva, cell sap, substances produced by reaction between the two, and the disintegrated remains of some of the cell walls." We have only found a similar condition of staining with safranin in the track of the stylets *after* their withdrawal (Plate XXXII, fig. 10), but from an examination of sections with the proboscis *in situ* it is noticeable that there is an absence of any granular substance at the margin of the stylets or even close to their tips in cells which are presumably subjected to sucking as soon as they are pierced (Plate XXXI, fig. 6). From Plate XXXII, figs. 9, 10, it will be seen that, although the cell walls collapse and tend to stain more deeply than the rest, the cell contents are not precipitated or at any rate no precipitate is disclosed by the safranin stain.

We are not, therefore, in a position to state whether the saliva is definitely toxic or plays only an indirect part in bringing about the initial collapse of the affected tissue. It is interesting to note that Butler⁽²⁾ states: "it would appear that the stimulus that causes the development of gall wood acts only for a time." That the irritant is diffusible and remains active for some time is proved by the fact that the cambium is stimulated to form gall wood for some weeks or even months.

CONTROL MEASURES.

It is obvious that the elimination or prevention of this form of tea canker depends entirely on the amount of control exercised over the mosquito bug. Unfortunately the insect is one that is extremely difficult to combat by mechanical or chemical measures. Although certain con-

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tact sprays are known to be effective in killing the pest, the quantity of the poison required to ensure an effective kill, coupled with the serious difficulties encountered in transporting and applying the materials throughout a tea garden, place such methods, generally, beyond the bounds of practical recommendation.

As has already been mentioned, however, there are certain ecological conditions under which the prevalence of the insect may be anticipated in definite areas. Amelioration of such conditions is the first essential in control. Secondly, given a normally abundant and cheap labour supply, concentrated hand collection of the insect in such areas, as a matter of estate routine, is undoubtedly effective. Tea nurseries, in addition to attention being paid to adequate drainage and sufficient clearing of the surrounding jungle, should be subjected to regular inspection at frequent intervals and hand-collecting gangs employed without delay whenever necessary. The same procedure should be adopted in any portion of a newly planted garden which, by reason of its situation, is liable to infestation by the insect. In Nyasaland such work is just as important as any other normal practice in the cultivation of tea.

SUMMARY.

1. Gnarled stem canker of tea is shown to be caused by the tea mosquito bug (*H. bergrothi* Reut.).
2. An account is given of the development of the canker from the time that the insect starts feeding.
3. The measurements of cankers found occurring in the field and those caused by different stages of the insect are given.
4. The path of the stylets in the tissues of the stem is described. The cause of the collapse of the affected tissues is discussed.
5. Control measures are considered in brief.

ACKNOWLEDGMENTS.

We are indebted to Dr E. J. Butler, Director of the Imperial Mycological Institute, for his valuable assistance received at various times. We wish to thank Prof. F. L. Engeldow for putting us in touch with Dr Wishart at Cambridge. Finally, we are grateful to Dr W. Small, Director of Agriculture, Nyasaland, for the facilities afforded to us during our investigations.

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EXPLANATION OF PLATES XXXI AND XXXII.

PLATE XXXI.

- Fig. 1. First stage of the disease. Cankers on green stem of tea.
- Fig. 2. The same cankers as in fig. 1 showing up more distinctly after scraping off the cortex.
- Fig. 3. Advanced stage of the disease. Many individual cankers with swollen callus lips forming the typical gnarled appearance of a diseased stem.
- Fig. 4. Russeted areas on stem and petiole of leaf caused by the insect feeding in the cortex. No cankers formed.
- Fig. 5. Usual feeding area of insect when sucking the youngest green stems. The cortical cells have collapsed outside the ring of thin-walled cells which are due to form the pericycle fibre ring. One week after feeding. No canker formed. (In all photographs the indications of the lettering are as follows: Co. = cortex; P.f. = pericycle fibre ring; P.p. = pericycle parenchyma; Ph. = phloem; Ca. = cambium; x. = xylem.)
- Fig. 6. Same type of feeding area as in fig. 5, 2 weeks after feeding. The pericycle fibre ring has commenced lignification thus separating the affected tissues from the vascular system. No canker formed.

PLATE XXXII.

- Fig. 7. *Development of canker (I).* Penetration of stem by stylets of mosquito bug. Tips of the stylets piercing the pericycle parenchyma. The barbed tips of the mandibles are easily recognisable.

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- Fig. 8. *Development of canker (II)*. $1\frac{1}{2}$ hours after feeding. Junction of healthy (*Hy.*) and diseased (*Dis.*) tissues. Pericycle parenchyma starting to collapse.
- Fig. 9. *Development of canker (III)*. 6 hours after feeding. Pericycle parenchyma collapsed. Phloem and cortex still healthy.
- Fig. 10. *Development of canker (IV)*. 24 hours after feeding. Pericycle and phloem collapsed. Cortex still healthy. (*St.*=stylet track.)
- Fig. 11. *Development of canker (V a)*. 1 month after feeding. Cortex still healthy and gall wood (*G.w.*) situated between necrotic tissues and old healthy wood.
- Fig. 12. *Development of canker (V b)*. 1 month after feeding. Cortex collapsed (normal bark formation). Gall-wood growth checked underneath the necrotic tissues but starting round the edge. Commencement of gnarled appearance of stem.

(Received January 31st, 1933.)



Fig. 1.



Fig. 2.



Fig. 3.



Fig. 4.

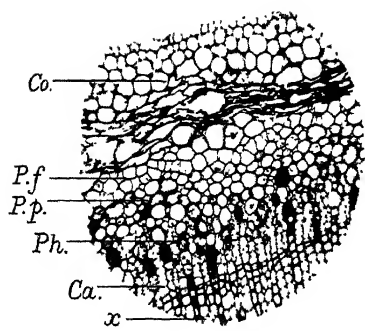


Fig. 5.

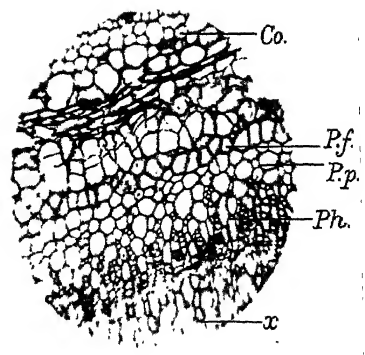


Fig. 6.

LEACH AND SMEE.—GNARLED STEM CANKER OF TEA CAUSED BY THE CAPSID BUG
(*HELOPELTIS BERGROTHI* REUT.) (pp. 691-706).

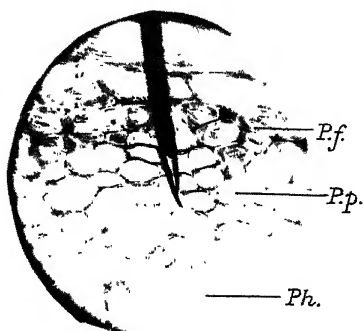


Fig. 7.

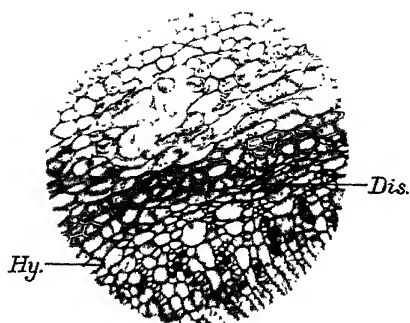


Fig. 8.

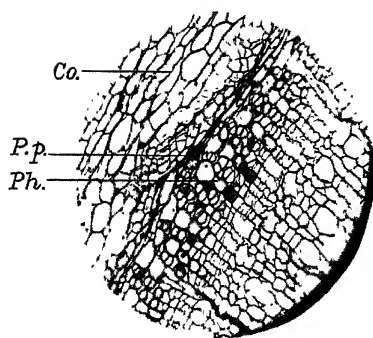


Fig. 9.

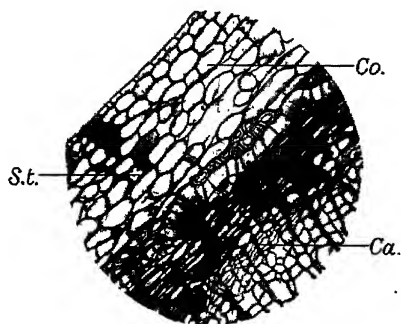


Fig. 10.

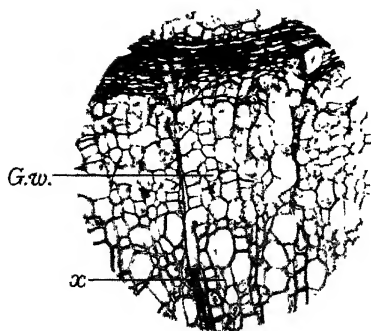


Fig. 11.

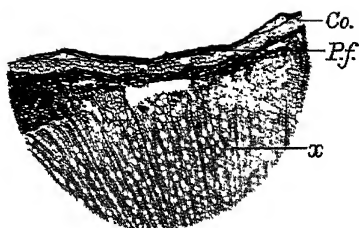


Fig. 12.

ON THE BIONOMICS AND STRUCTURE OF SOME DIPTEROUS LARVAE INFESTING CEREALS AND GRASSES

I. *OPOMYZA FLORUM* FABR.

By I. THOMAS, M.Sc.

(*School of Agriculture, Cambridge.*)

(With 8 Text-figures.)

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I. INTRODUCTION.

Opomyza florum Fabr. as a pest of cereals has been recorded chiefly from Russia. In 1914 Dobrovliansky(2) recorded the larva as being injurious to wheat and in 1915 Kulagin(6) found it attacking rye and barley. Subsequent records from wheat are those of Pisnyachevskii(8) and Ruszkowski(9) in 1927, and Kreiter(5) in 1928. The biology of the species has been studied by Bielsky(1), who gives its life-history and figures the anterior spiracles and cephalo-pharyngeal skeleton of second- and third-instar larvae. Bielsky's account has been summarised by Czerny in Linder's *Die Fliegen der Palaearktischen Region*(7).

The first record of a species of *Opomyza* injurious to wheat in England is by Taylor(10) in 1931. He states: "*Wheat*. Twelve acres following seeds have been ruined on the wolds by the combined attacks of *Frit*, *Opomyza*

sp. and *Longitarsus ochroleucus*." Although it is probable that the report refers to an attack by *O. florum*, specimens of the larvae could not be obtained, so that the species could not be identified.

Injury to wheat occurred on experimental plots at the University Farm, Cambridge, in April 1931. Several varieties of wheat were affected and on examination damaged tillers, which showed symptoms similar to an attack by *Leptohylemyia coarctata* Fall., were found to harbour first- and second-instar larvae of *O. florum*. Infested tillers were collected and planted in pots in the greenhouse, and others were caged in the open. Records of times of emergence of the flies were taken from the cages and by sweeping in the field. For observation on oviposition, flies were caged in hurricane lamp glasses with muslin tops, placed over seedling cereals and grasses.

II. BIONOMICS.

(a) *Field observations.*

In 1931 first-instar larvae were found from April 14th to April 20th, second-instar larvae from April 14th to the 24th, and third-instar larvae from April 20th to May 24th. Unhatched puparia were found from May 10th to June 14th.

In 1932 first-instars were found as early as April 2nd and unhatched puparia until June 15th.

Adults live from June until November. In 1931 from sweepings taken in meadows and pastures only very few specimens of adults were obtained from the end of June until mid-August, but after this date larger numbers were taken especially along the hedgerows. Dobrovliansky (2) found them mostly on blossoming peas and in lucerne fields during August and September. Bielsky (1) found that during hot weather they frequented woods and shaded places, returning to the fields in autumn. Bielsky's findings seem to hold good for this country, for in 1932 during June and July sweepings were taken in more shaded places, chiefly along wood-sides and along hedgerows of clover and sainfoin fields when a number of adults were caught.

Adults taken during the summer of 1931 were caged in the greenhouse over young wheat plants and various grasses. Eggs were not laid until October 18th. Flies caught during October and early November continued to oviposit until November 20th. The last date in which adults were caught in the field was November 15th.

In the laboratory eggs were laid on the soil near sprouting wheat plants and, since first-instar larvae were not found until the spring, it was

concluded that the species probably overwintered in the egg stage. The time and position of egg-laying suggests that early sown winter wheat would be more heavily infested than that sown after the middle of November. This would be in keeping with an observation made by Pisnyachevskii (8), who states that the species appeared to cause more damage to the earlier sown varieties of winter wheat.

During the third week of April, 1932, an examination was made of plots of wheat at the University Farm, Cambridge, which had been sown at different dates in the preceding autumn. The various plots and their dates of sowing are shown in Table I. There were about 150 rows per plot, and about 23 plants per row.

Table I.

Dates of sowing of plots of wheat at the University Farm, Cambridge, in 1931.

Plot No.	Date of sowing	Plot No.	Date of sowing
1-10	Oct. 15th	18-22	Oct. 27th
11	" 16th	23	" 31st
12	" 19th	27	Nov. 2nd
13	" 20th	38-43	" 24th
14-17	" 26th		

Of the plots enumerated in Table I Nos. 1, 3, 5, 13, 18, 19, 23, 27, 38, 41, 42 and 43 were examined for infestation by larvae of *O. florum* (certain plots were planted for special purposes and were not available for examination). The number of infested tillers in 10 rows per plot (rows were taken at random) were counted and the results are summarised in Table II.

Table II*.

Number infested with larvae of O. florum in wheat plots sown at different dates on the University Farm, Cambridge.

Plot No.	Date of sowing	Number of infested plants per row (23 plants per row)	Total	Plants infested %
1	Oct. 15th	7, 9, 6, 2, 5, 6, 10, 6, 8, 6	65	28.3
3	" 15th	6, 3, 8, 5, 5, 2, 5, 3, 9, 6	52	22.6
5	" 15th	3, 4, 7, 10, 3, 5, 5, 5, 7, 4	53	23.0
13	" 20th	3, 2, 2, 6, 2, 3, 2, 3, 3, 5	31	13.5
18	" 27th	2, 2, 1, 0, 0, 3, 1, 0, 2, 2	13	5.7
19	" 27th	3, 1, 1, 1, 0, 2, 0, 0, 3, 3	14	6.1
23	" 31st	4, 2, 4, 3, 1, 1, 0, 3, 0, 2	20	8.7
27	Nov. 2nd	1, 0, 0, 0, 2, 0, 1, 0, 1, 0	5	2.2
38	" 24th	0, 0, 0, 0, 0, 0, 0, 1, 0, 0	1	0.4
41	" 24th	0, 1, 0, 0, 0, 0, 0, 0, 0, 0	1	0.4
42	" 24th	0, 0, 0, 0, 0, 0, 0, 0, 0, 0	0	0
43	" 24th	0, 0, 0, 1, 1, 0, 0, 0, 0, 1	3	1.3

* The figures given in this table were submitted to Dr Wishart who found that for the purposes of these counts the material was homogeneous.

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Table II shows that the earlier sown plots were more heavily infested than the later ones, but even Plot 43 which was sown on November 24th (wheat would not be through the ground for at least a week) had three tillers infested. This has two possible explanations, either some adults must have been present as late as December 1st or eggs must have been laid on a bare fallow.

The highest percentage of plants infested was 28.3, but since there was an average of more than six tillers per plant the injury incurred was not serious.

In Table II it will be noticed that a low count of "2" was obtained in Plot 1. This count was obtained from a row of the variety Old Fashioned II. Plot 1 was planted as follows:

	Rows
Vulgare PP	3
Old Fashioned IX	10
Vulgare PP	2
Old Fashioned IV	10
Vulgare PP	2
Old Fashioned II	10
Vulgare PP	2
Square Heads Master	10
Vulgare PP	2
Red Stettin B	10
Vulgare PP	2
Sun III	10
Vulgare PP	2
Iron III	10
Vulgare PP	2
Temperley 01036 B	10
Vulgare PP	3

A close examination of the plot revealed that the variety Old Fashioned II did not seem as heavily infested as any of the other varieties; a count was therefore taken of the infested plants in 10 rows of the variety and 10 rows of the variety Vulgare PP. The figures obtained were as follows:

Vulgare PP	5, 8, 3, 6, 11, 9, 13, 10, 10, 9	Average 8.4
Old Fashioned	3, 5, 5, 3, 2, 0, 3, 1, 3, 5	" 3.0

The difference between the infestation of Vulgare PP and Old Fashioned¹ II is significant, so that the latter variety may be said to be more resistant to infestation by *O. florum* than the former.

(b) *Laboratory observations.*

Larvae were collected in May, 1931, and fed on agar medium consisting of malt extract 0.5 per cent., agar 2.5 per cent., water 95 per cent., as prepared by Harris (3). The first larvae pupated on May 8th. When pupating the larva retracts its head as far as possible into the pro-

¹ On the University Farm this variety is resistant to *Puccinia glumarum* and to low temperatures."—Note by Prof. Engledow.

thoracic segment so that the anterior spiracles project away from the surface of the body. The colour changes very gradually; after 6 hours quiescence only a faint yellowing of the chitin is seen and the puparium reaches its normal brown colour after 24–36 hours. The pupal period in the laboratory varied from 19 to 25 days and in the open from 19 to 26 days. The first adults hatched from out-door cages on June 6th in 1931 and on June 11th in 1932. Although adults thus hatched were provided with sugar solution and flowers they could not be kept alive until oviposition took place, 3 weeks being the longest period in captivity. To

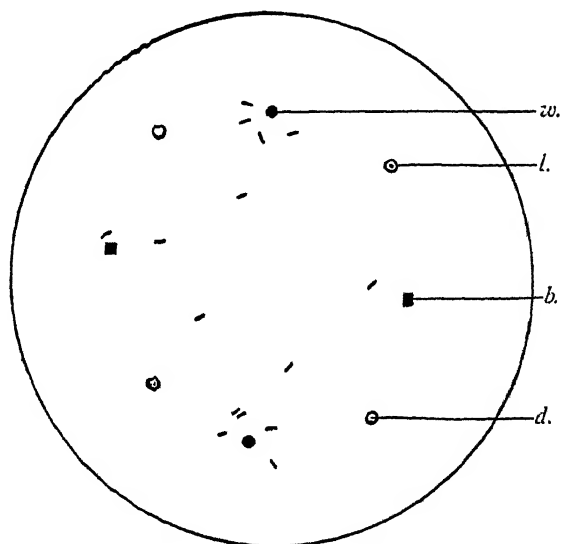


Fig. 1. Showing position of eggs laid by *O. florum*. w. wheat; b. barley;
l. *Lolium perenne*; d. *Dactylis glomerata*.

obtain the date of oviposition and further information on the various instars, adults which were swept in the fields from July to October were caged in various grasses and cereals in the greenhouse.

Mating took place in October and in both 1931 and 1932 the first date on which eggs were found was October 18th.

Adults were caged in hurricane lamp glasses over pots as previously described, each pot being planted with two seedlings of each of the following: wheat, barley, *Dactylis glomerata* and *Lolium perenne*.

Eggs were laid on the soil chiefly near the wheat seedlings. Fig. 1 gives the position of the eggs laid in a cage examined on October 23rd in

1931. It will be noted that they are laid mainly within 1 in. of the wheat plants, occasional eggs being fairly near the barley seedlings.

First-instar larvae were found in the wheat and barley seedlings on December 2nd. The duration of the various instars in the laboratory was as follows:

				Days
Egg	45-52
First-instar	3-6
Second-instar	2-5
Third-instar	23-30

The duration of the various instars and the pupal period corresponds closely with that observed in the field; the hatching period of the egg, however, is much shorter in the laboratory, for eggs laid in the field remain unhatched over the winter.

III. STRUCTURE.

(a) *The third-instar larva.*

(i) *Morphology.*

The *third-instar larva* is shining creamy-white in colour and varies in length from 6.8 to 7.8 mm. and in width from 0.8 to 1.2 mm. It consists of a head, three thoracic and eight abdominal segments and tapers gradually to a point anteriorly and has a truncated posterior end.

The *head* (Fig. 2) is rounded and is capable of almost complete retraction within the prothoracic segment. Ventrally it is divided by a shallow depression extending from just behind the antennae into the mouth.

The *antennae* (*a*) are situated close together on the anterior margin of the head; each consists of two joints, a small bullet-shaped distal joint fitting into a short broad basal joint.

The *maxillary palpi* (*m.p.* and fig. M) are comparatively large and situated a short distance behind the antennae. Each is raised on a slight prominence and consists of a number of minute sensory papillae placed within a chitinous ring (*r*). Two minute sensory papillae occur near the outer margin, and one near the inner margin of each ring. A second pair of papillae occurs behind the maxillary palpi; these are in a position similar to those in the larva of *Drosophila* sp., which Keilin⁽⁴⁾ has shown to be supplied by the same nerve as the maxillary palpi, and regards as a second part of the maxillary palpi themselves; each is slightly raised and bears two minute papillae. A minute sensory papilla (*s.p.*) is also present on either lateral margin of the head. The region of the head behind the

maxillary palpi is divided into small areas separated by irregular shallow channels. A few of these areas on either side bear minute denticles or teeth.

The *labium* (*l.*) is a small heart-shaped structure on the ventral surface of the head immediately below the anterior margin of the dentate sclerite; it bears near its posterior border two sensory areas each having two minute papillae—the labial palpi (*l.p.*).

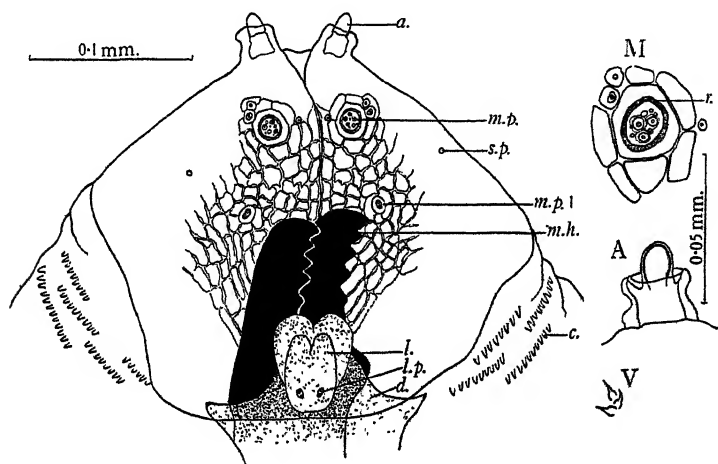


Fig. 2. Ventral view of head of third-instar larva of *O. florum*. *a.* antenna; *m.p.* maxillary palp; *s.p.* sensory papilla; *m.h.* mouth hook; *l.* labium; *l.p.* labial palp; *d.* dentate sclerite; *c.* chitinous denticles. *M.* Maxillary palp. *r.* chitinous ring. *A.* Antenna. *V.* Vestigial leg of prothoracic segment.

Table III.

Number of rows of denticles on the anterior margins of the segments of the larva of O. florum.

Segment		No. of rows of denticles		
		Dorsal	Ventral	Lateral
Thoracic	I	19	10	12
"	II	11	10	9
"	III	6	6	8
Abdominal	1	3	4	3
"	2	3	4	3
"	3	2	3	2
"	4	2	3	0
"	5	2	3	0
"	6	1	2	0
"	7	0	2	0
"	8	0	2	0

Each thoracic segment bears on its ventral surface and near its anterior margin a transverse row of six minute sensory organs, and also a pair of vestigial legs (Fig. 2) near the middle of the segment, placed one on either side near the mid-ventral line of the body. These are homologous with those described by Keilin (4), each vestige consisting of three minute bristles each having a small basal swelling.

The anterior margin of each segment bears a number of incomplete rings of minute chitinous denticles (*c.*), these are larger on the thoracic than on the abdominal segments. Their arrangement may be visualised from Table III.

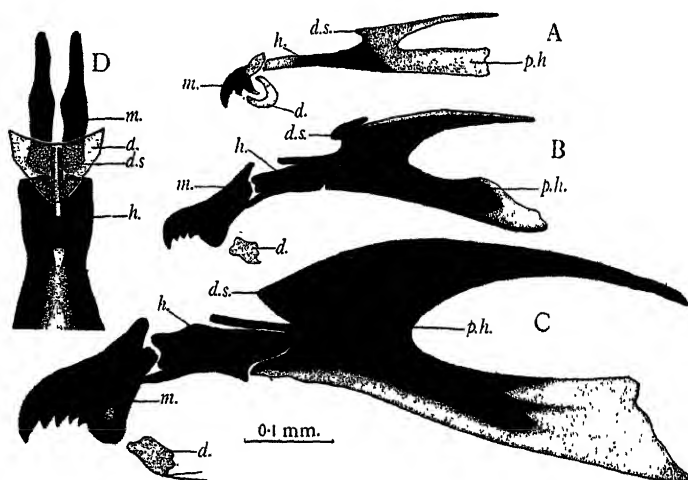


Fig. 3. Cephalo-pharyngeal skeletons of various instars of larva of *O. florum*. A. First-instar larva. B. Second-instar larva. C. Third-instar larva—lateral view. D. Third-instar larva—ventral view. *m.* mouth hook; *h.* hypostomal sclerite; *d.s.* dorsal sclerite; *p.h.* pharyngeal sclerite; *d.* dentate sclerite. (In A *h.* = hypostomal region, and *p.h.* pharyngeal region.)

(ii) *The cephalo-pharyngeal skeleton* (Fig. 3 C).

The *cephalo-pharyngeal skeleton* is built up of a pair of *mandibular sclerites* or *mouth hooks* (*m.*), articulating with a *hypostomal sclerite* (*h.*) which is attached on either side to a large *pharyngeal sclerite* (*p.h.*). The latter are fused dorsally to a *dorsal sclerite* (*d.s.*) and there is also a *median ventral* or *dentate sclerite* (*d.*).

The *mouth hooks* are heavily sclerotised and each has a large apical tooth followed by four smaller teeth and a large rounded ventral projection. They are not fused together, but articulate separately with the

hypostomal sclerite by means of two sclerotised rods which project from, and are attached to, the cross-piece of the hypostomal sclerite.

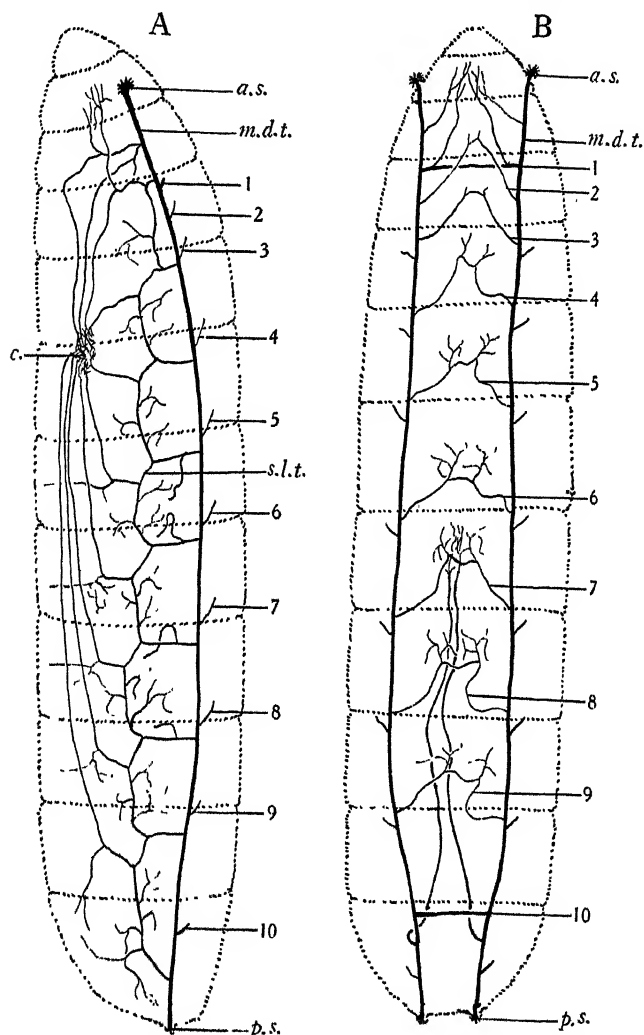


Fig. 4. Respiratory system of third-instar larva of *O. florum*. A. Lateral view. a.s. anterior spiracle; m.d.t. main dorsal tracheal trunk; 1-10, dorsal transverse commissures; c. concentration of tracheoles at "brain"; s.l.t. secondary longitudinal tracheal trunk; p.s. posterior spiracle. B. Dorsal view. Lettering as for A.

The *hypostomal sclerite* is H-shaped and heavily sclerotised; each posterior projection of the H is attached to a large pharyngeal sclerite.

Each *pharyngeal sclerite* has an anterior projection which extends over the posterior projection of the hypostomal sclerite; posteriorly it bifurcates to form dorsal and ventral projections.

The *dorsal sclerite* is lightly sclerotised and is fused on either side to the anterior regions of the pharyngeal sclerites.

The *median ventral* or *dentate sclerite* consists of a V-shaped lightly sclerotised plate situated beneath the point of articulation of the mandibular with the hypostomal sclerite.

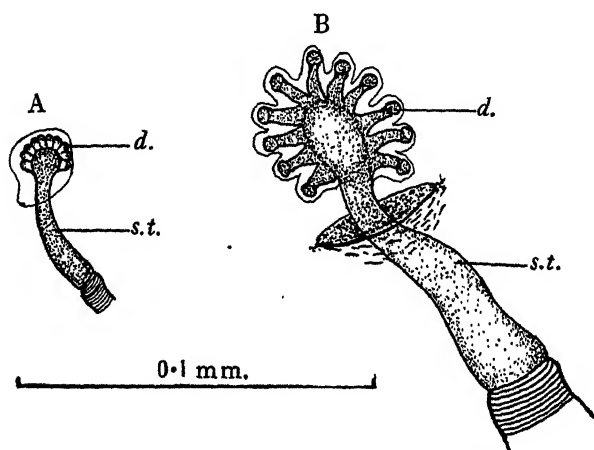


Fig. 5. A. Anterior spiracle of second-instar larva of *O. florum*. B. Anterior spiracle of third-instar larva. *d.* digitate process; *s.t.* stigmatic trunk.

(iii) *The respiratory system* (Fig. 4).

In this instar the larva is amphipneustic. The *anterior spiracles* (Fig. 5 B) are comparatively small; each opens to the exterior on the latero-dorsal surface, near the posterior margin of the prothoracic segment. The *stigmatic trunk* (*s.t.*) opens anteriorly in a number of digitate processes each having a small ring of chitin—the *peritreme* at its distal end which surrounds a *spiracular cleft*. The number of *digitate processes* (*d.*) vary from nine to eleven, the usual number being ten. Two main *dorsal tracheal trunks* on either side of the body connect up the anterior with the posterior spiracles.

The *posterior spiracles* (Fig. 6) open out on short *stigmatic papillae* on the truncated end of the last abdominal segment; each consists of a *stigmatic trunk* (*s.t.*) which divides into three short branches. These open on to the *stigmatic plate* (*s.p.*), each through an oval aperture—the

spiracular cleft (*s.l.*), which is surrounded by a chitinous ring—the *peritreme* (*p.*). Radiating from the stigmatic plate are four series of chitinous branching hair-like structures (*b.h.*).

The *main dorsal tracheal trunks* (Fig. 4) are united to one another by a series of ten *dorsal transverse commissures* (1–10), the first in the meta-

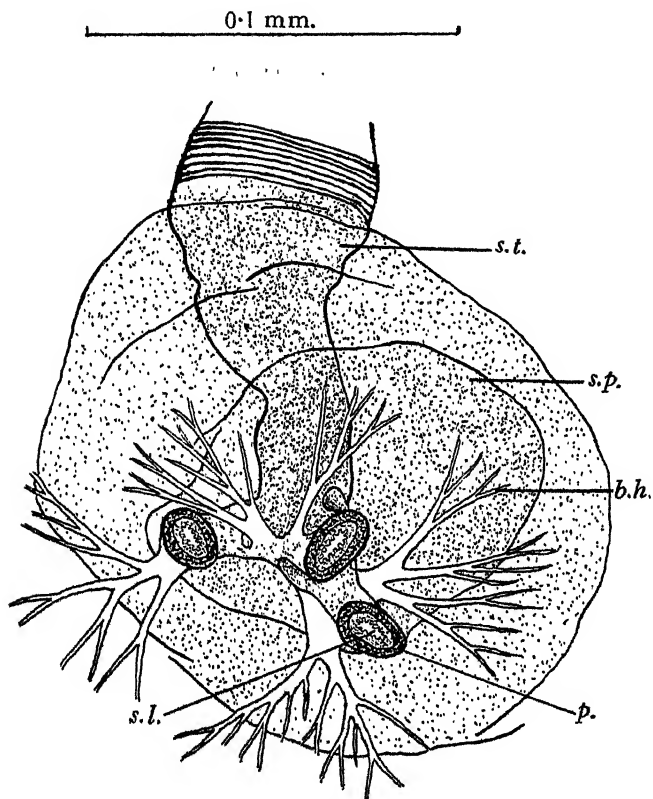


Fig. 6. Posterior spiracle of third-instar larva of *O. florum*. *s.t.* stigmatic trunk; *s.p.* stigmatic plate; *b.h.* branching hairs; *p.* peritreme; *s.l.* spiracular cleft.

thoracic segment and the last in the last abdominal segment. The first and last commissures are straight and much thicker than the others, a pair of branches arise from the former which go forward to supply the cephalo-pharyngeal mass. The second and third commissures loop forward and give off a pair of unbranched tracheoles, the fourth to the ninth commissures inclusive also loop forward and give off a pair of tracheoles which are finely branched.

There is a *secondary latero-ventral longitudinal trunk (s.l.t.)* which is connected to the main trunk by branches which arise from the latter immediately posterior to the origins of the transverse commissures. Each section of the secondary trunk gives off a branch which divides to give a ventral branch and a branch which passes forward to supply the ventral ganglion. From the main tracheal trunk in the thoracic segments branches arise which supply the cephalo-pharyngeal mass, these also give off tracheoles which pass posteriorly to the ventral ganglion.

(b) *The second-instar larva.*

The *second-instar larva* varies in length from 2.7 to 3.5 mm. and in width from 0.4 to 0.6 mm. Except that the various structures are smaller it is in most respects very similar to the third-instar larva. The sensory organs on the head and thoracic segments are all homologous with those of the third instar; the denticles at the junctions of the body segments are also similar, but there are fewer rows.

The *cephalo-pharyngeal skeleton* (Fig. 3 B) is built up of sclerites homologous with those of the third instar; there is a slight difference in the structure of the *mouth hooks (m.)*, each of which has four instead of five teeth; the *dorsal sclerite (d.s.)* is also comparatively smaller and is not as firmly fused to the *pharyngeal sclerites*.

Another difference from the third-instar larva occurs in the structure of the *anterior spiracle* (Fig. 5 A); in this instar it is very small, each *digitate process (d.)* being very short and closely applied to its neighbour so that, as in the second instar larva of *B. tripunctata*, it has the appearance of a transverse section through a rosette. The number of digitate processes corresponds to the number in the spiracle of the third instar.

(c) *The first-instar larva.*

The *first-instar larva* varies in length from 1.2 mm. when newly hatched to 2.5 mm. before ecdysis. Immediately after hatching, it is semi-translucent and tapers gradually from the metathoracic segment to the truncated posterior end. Except that in this instar the larva is metapneustic, its morphological details are very similar to those of the second- and third-instar larvae.

In the *cephalo-pharyngeal skeleton* (Fig. 3 A), however, there is no differentiation into *hypostomal (h.)* and *pharyngeal sclerites (p.h.)*. The *mouth hooks (m.)* are comparatively large and have a large serrated apical tooth and two smaller teeth; each articulates with a sclerotised

rod which corresponds to the anterior projection of the hypostomal sclerite of the second- and third-instar larvae but there is no cross-piece. Each rod extends posteriorly to form a *pharyngeal portion* which bifurcates into dorsal and ventral projections. The pharyngeal portions are connected dorsally and anteriorly by a lightly sclerotised narrow strip which is homologous with the dorsal sclerite of the later instars.

There are no anterior *prothoracic spiracles*. The *posterior spiracles* are very small and each opens out through two spiracular clefts on the posterior end of the last abdominal segment. There is a *stigmatic plate* from which arise branched hairs as in the later instars. From the posterior spiracles two main dorsal tracheal trunks extend forward and terminate in tracheoles in the region of the thoracic segments. As in the second and third instars there are ten *dorsal transverse commissures* and branches which pass forward ventrally to supply the brain.

(d) *The egg.* (Fig. 7.)

When laid, the egg of this species is about 0.57 mm. long and about 0.19 mm. in diameter; it is white in colour and tapers towards both ends. Its ventral surface is flattened so that the dorsal surface is considerably curved, but this curvature is not so pronounced immediately before the larva hatches when the egg may attain a length of 1.14 mm. The chorion is raised in a number of longitudinal ridges extending from end to end of the egg, with occasional ridges ending abruptly. It is thickened at both ends and the anterior end has a distinct plug-shaped micropylar area (*m.*). Its whole surface is raised into small bead-like papillae.

(e) *The puparium.* (Fig. 8.)

The cylindrical puparium is brown in colour and is constructed from the hardened integument of the third-instar larva. It varies from 4.0 to 4.4 mm. in length and is about 1.0 mm. in diameter. The morphological characters of the larva including the segmentation and denticles are retained and the larval spiracles (*a.s.* and *s.p.*) are present on the anterior and posterior end respectively. The cephalo-pharyngeal skeleton (*c.p.s.*) is visible through the chitin at the anterior end where it lies on the inside of the ventral surface of the puparium. The dorsal region of the thoracic segments is flattened and slopes from the dorsal to the ventral surface forming an angle of about 45°.

On emergence of the adult a longitudinal slit occurs on either side of the thoracic segments, extending as far as the anterior margin of the

first abdominal segment where a transverse slit occurs which may extend almost to the mid-dorsal line.

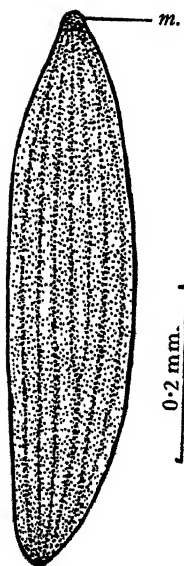


Fig. 7. Egg of *O. florum*.

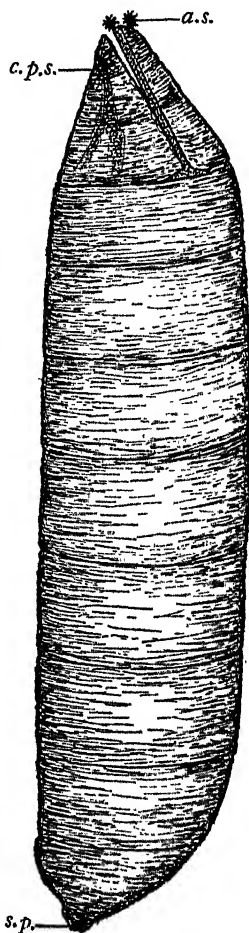


Fig. 8. Puparium of *O. florum*. *c.p.s.* cephalopharyngeal skeleton; *a.s.* anterior spiracle; *s.p.* stigmatic papilla.

IV. SUMMARY.

O. florum Fabr. as a pest of cereals has been recorded chiefly from Russia. Injury to wheat by the larva of the species occurred on the experimental plots at the University Farm, Cambridge, in 1931 and 1932.

Eggs are laid near sprouting wheat in the autumn; these hatch in the following April and infest the young plants, the central shoot being severed. There are three larval instars and pupation takes place inside the plants in May. Adults emerge in June and live until November. They frequent shaded places and may be caught along hedgerows of clover and sainfoin fields.

Early sown winter wheat is more liable to attack than that sown after the end of October.

The variety Old Fashioned II was found to be more resistant to attack than other varieties examined.

A description is given of the egg, the larval instars and the puparium.

The writer is indebted to Mr F. R. Petherbridge for supervision of the work, and to Mr J. E. Collin for identification of the species.

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ON *HOPLOCAMPA FLAVA* L., THE PLUM SAWFLY

BY HERBERT W. MILES, M.Sc., Ph.D.

(Victoria University of Manchester)

I. THOMAS, M.Sc. AND G. L. HEY, B.A.

(School of Agriculture, Cambridge.)

(With Plates XXXIII and XXXIV and 6 Text-figures.)

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In a previous paper (1) an account has been given of the occurrence of *Hoplocampa flava* L. in Britain, and the biology and economic importance of the insect in the fruit-growing districts of East Anglia has been discussed. The present paper deals with the distribution of the species; descriptions of the immature stages are amplified and further particulars are given regarding the development of the insect.

I. IDENTITY AND DISTRIBUTION.

Enslin (5) uses the name *H. flava* L. for the yellow-bodied sawfly with infuscated wings that infests *Prunus* spp., and gives as synonyms *ruficapilla* Gmel., *ferruginea* F., *simplex* Fall., *brunnea* Klug, *verticata* Lep., and *apicaris* W. F. Kirby. Morice (8) refers to *H. ferruginea* Panz. and includes it in his key under the name that Dalla Torre (4), like Enslin, lists as a synonym of *H. flava* L.

A species of plum sawfly, sometimes referred to as *H. ferruginea*, has been recorded from Surrey (7); Kent, Sussex, Cornwall, Lincolnshire, Nottinghamshire, Staffordshire, Herefordshire and Westmorland (13); Norfolk (3); Worcestershire, Gloucestershire and Northamptonshire (9); Lancashire and Cheshire (2); Yorkshire (1); Cambridgeshire and Huntingdonshire (11), and Devonshire (10). From this it is apparent that the species is generally

distributed in Britain and may cause injury in any area where plums and damsons are grown extensively.

II. DESCRIPTION OF ADULTS (Plate XXXIII, fig. 1) AND COMPARISON WITH OTHER BRITISH HOPLOCAMPIDS.

The head and mesonotum are brownish-yellow, strongly punctured and rather dull. The abdomen above and below is yellowish and somewhat shining. The antennae and legs are yellow, darkening a little after death. The fore wings are infuscated on the basal two-thirds, the coloration being most distinct transversely across the middle of the wing. The apical third of the wing is practically clear. The veins and stigma are yellow, the stigma being slightly darker basally. The hind wings are clear. Length: ♀♀ 4.5-5.5 mm.; ♂♂ 4.5-5 mm.

In both sexes the metathorax is largely black. The male has the following conspicuous black markings on the mesothorax: a somewhat triangular area on the median lobe, a longitudinal band on each of the lateral lobes of the mesonotum, and a large area on the disc extending from the apex of the median lobe backwards to the apex of the scutellum. Females may be tinged with black near the longitudinal furrow of the median lobe of the mesonotum. In the male the ocellar region of the head is conspicuously black, while in the female the ocelli may be narrowly ringed with black.

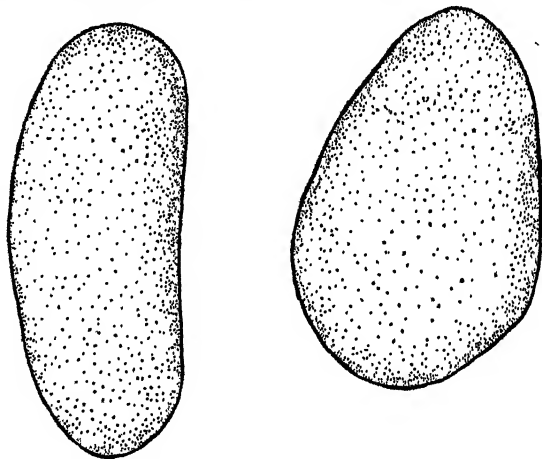
The lancets of the ovipositor are long and slender and compoundly dentate, each of the major teeth bearing 3-5 denticles. The apical 6-7 teeth are well developed and are directed forwards. The sheath is stout and has clearly defined transverse, chitinous ridges (Plate XXXIII, fig. 2).

H. flava L. is readily distinguished from the seven species of *Hoplocampa* recorded as British. It is the only yellowish-brown species with infuscated wings (Plate XXXIII, fig. 1). *H. rutilicornis* Kl. and *H. chrysorrhoea* Kl. are on the wing at the same time and may occur in the blossoms of *Prunus* spp. These species are smaller than *H. flava* and are marked with black on the abdomen. *H. crataegi* Kl. bears some resemblance to *H. flava* though it is slighter and is more shining and less coarsely punctured on the mesonotum, but it can be readily distinguished by its hyaline wings. *H. pectoralis* C. G. Thoms., which often occurs in company with *H. crataegi*, is also slighter than *H. flava*, has more black about the head and thorax and has hyaline wings. The flight period of *H. crataegi* and *H. pectoralis* is nearly two months later than that of *H. flava*. *H. testudinea* Kl., which infests apples⁽⁶⁾, is much larger than

H. flava and is altogether darker, and *H. alpina* Zett., associated with mountain ash, is much smaller than *H. flava* and is very pale with hyaline wings.

III. LIFE HISTORY AND DESCRIPTION OF STAGES.

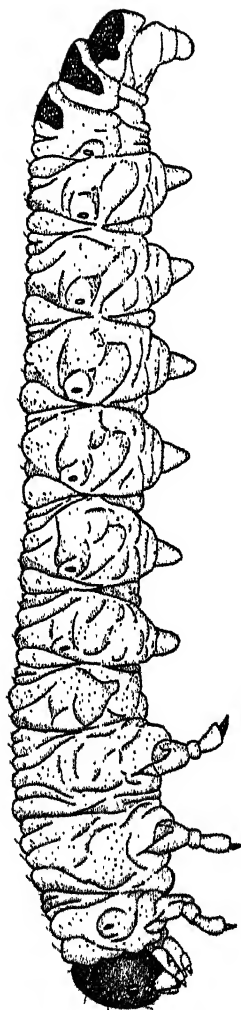
Petherbridge, Thomas and Hey (11) have shown that *H. flava* is on the wing at the time the plums are in blossom. Eggs are laid on the calyx or torus, and on hatching the larvae enter the developing fruit, where their feeding causes the fruit to fall prematurely. Mature larvae construct cocoons in the soil. They spend the winter as larvae in the cocoons and pupate in the early spring. *H. flava* is univoltine.



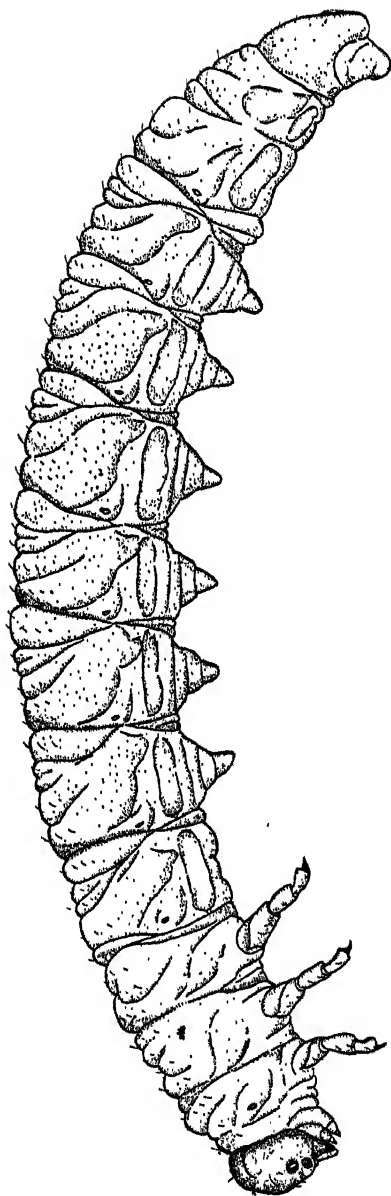
Text-fig. 1. Eggs of *H. flava* L. dissected out from plum blossom,
May 2nd, 1932. $\times 86$.

The egg. The egg is inserted in the calyx of the blossom. When first laid it is elongate oval, but after a few days it swells considerably and the shape may alter. Text-fig. 1 gives outline drawings of two eggs to show the variation in shape and Plate XXXIV, fig. 3, shows an egg partly dissected out of the calyx. Eggs dissected out of calyx tissue measured 0.62–0.67 mm. in length, and varied from 0.25 to 0.30 mm. in width.

The larva. The larva (Text-figs. 2, 3) of *H. flava* is fairly typical of the genus. The head (Text-fig. 4) is sub-globose and from the front appears roughly circular, with microscopic setae laterally on the epicranium and on the frons. The frons is pentagonal. The clypeus is short and broad. The labrum is narrowly oval and emarginate on its lower edge. The epipharynx is attached to the labrum and bears two groups of stoutish

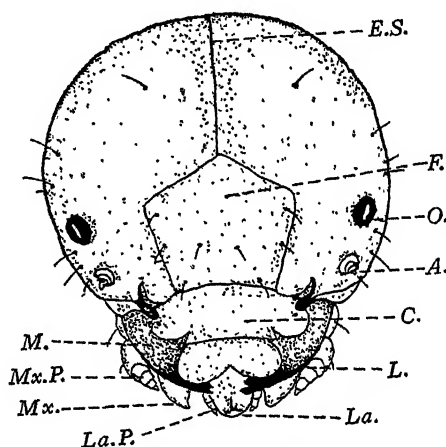


Text-fig. 2. Larva of *H. flava* in fourth instar. $\times 14$.



Text-fig. 3. Larva of *H. flava* in fifth instar. $\times 16$.

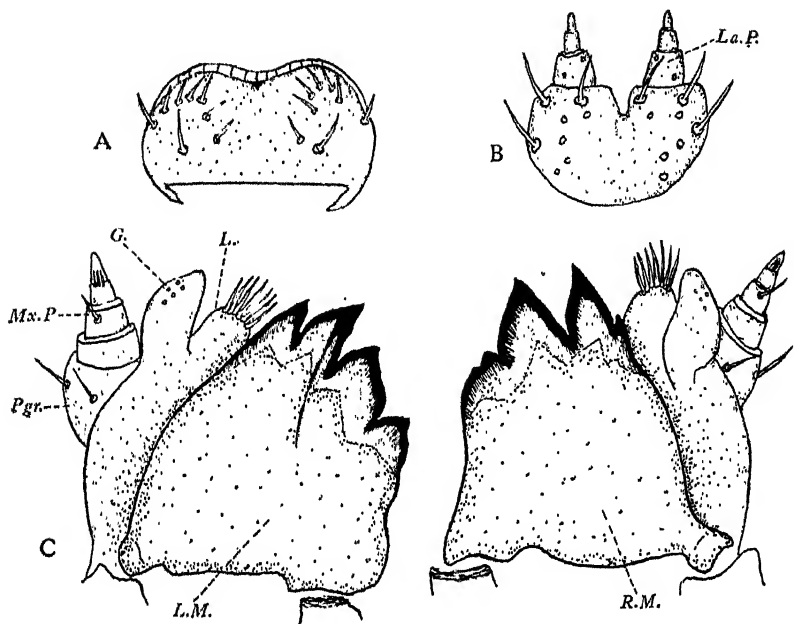
setae (Text-fig. 5 A). The eyes are dark and conspicuous and the antennae (Text-fig. 6) are so flattened that the segments are not easily distinguished. The mandibles are thick, strongly chitinated and sharply dentate. They are dissimilar, the dentations of the left mandible being larger than those of the right. The maxillae (Text-fig. 5 C) are fleshy and prominent. The galea and lacinia are readily distinguished; the galea is conical and the lacinia bears conspicuous setae. There is a broad palpiger bearing a four-segmented palp. The labium (Text-fig. 5 B) is fleshy and on its lower surface bears several stout setae. The labial palps are three-segmented.



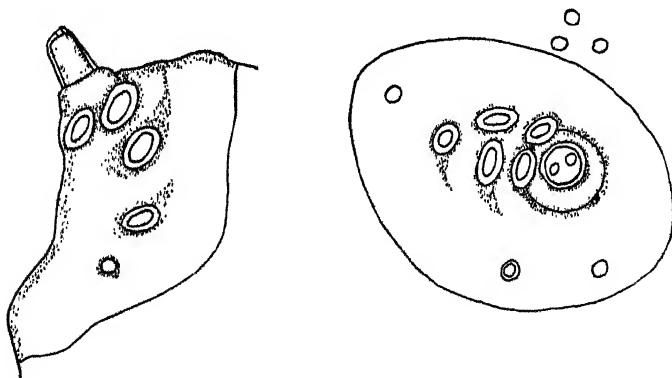
Text-fig. 4. Head of larva of *H. flava*, frontal view. $\times 48$. E.S.=epicranial suture; F.=frons; C.=clypeus; L.=labrum; M.=mandible; Mx.=maxilla; Mx.P.=maxillary palp; La.=labium; La.P.=labial palp; O.=ocellus; A.=antenna.

The thoracic and abdominal segments are annulated, the annulations of abdominal segments 2-7 appearing constant. The setiferation is obscure. The spiracles are prominent and occur on the prothorax and on abdominal segments 1-8 inclusive. The thoracic legs are long with well-marked articulations and terminate in a brownish fused tarsus and tarsal claw. Prolegs are present on abdominal segments 2-7 and 10. The suranal lobe is prominent in all the instars. The caudal tergites are chitinated in some instars, the chitin appearing darkest just prior to ecdysis.

First-instar larva. The head and tergites of segments 12 and 13 are yellow to yellowish-brown, the eyes are dark brown and conspicuous, and the body and legs creamy white.



Text-fig. 5. Mouth parts of *H. flavu*. $\times 110$. A. Ventral view of labrum with epipharyngeal setae. B. Ventral view of labium with labial palps. C. Mandibles and maxillae. L.M. = left mandible; R.M. = right mandible; Pgr. = palpiger; G. = galea; L. = lacinia; La.P. = labial palp; Ms.P. = maxillary palp.



Text-fig. 6. Antennae of *H. flavu* as seen from the side and from above. $\times 800$.

Second-instar larva. The head and tergites of segments 12 and 13 are yellow to yellowish-brown, but the head is more shining than in the previous instar and the dark dorsal sclerites are more extensive. The eyes are dark and the mandibles brown.

Third-instar larva. The head and dorsal sclerites of segments 12 and 13 are yellowish-brown, and in this instar there is a narrow yellowish dorsal sclerite on segment 11. The eyes, mandibles and tarsal claws are dark.

Fourth-instar larva. The head and tergites of segments 11, 12 and 13 are bright yellow to yellowish-brown. The eyes and mandibles are dark.

Fifth-instar larva. The head is yellow to pale orange, noticeably paler than in the fourth instar. The tergites of the terminal segments are also paler except at the margins of tergites 12 and 13. The eyes and mandibles are brownish and the body creamy and more elongate.

IV. INCUBATION PERIOD AND LARVAL DEVELOPMENT.

Observations indicate that the incubation period varies greatly. Sprengel⁽¹²⁾ records that it varies from 4 to 14 days, depending on weather conditions, and quotes similar variation from the records of other workers: Ritzema Boz giving 8-24 days, Theobald 7-14 days and Fintescue 6-12 days. Observations made over several years⁽¹¹⁾ show that almost a month passes between the first appearance of the adults and the finding of the first larvae, and this suggests that under English conditions the incubation period is about three weeks.

In 1932 observations were made on the duration of larval life. Plums examined on May 21st revealed thirteen larvae all in the first instar. On May 29th four first-instar larvae and twenty-two second-instar larvae were found. On June 5th there were only three second-instar larvae, eighteen third-instar larvae and two fourth-instar larvae. On June 11th five third-instar larvae and twelve fourth-instar larvae were collected. On June 22nd the fourteen larvae collected were all in the fifth instar and were feeding actively. On June 25th thirteen fifth-instar larvae were collected; these appeared fully fed and were leaving the plums. These observations indicate that the larval feeding period is about 36 days: 8-9 days in the first instar, 6-7 days in the second, 6-7 days in the third, 6-8 days in the fourth and 6-7 days in the fifth. Sprengel⁽¹²⁾ has shown that in Germany the feeding period may be shortened to 30 days or prolonged to over 40 days as a response to weather conditions.

Records of the width of the head in the different larval instars were kept in order to check the number of instars and give data on the rate of growth.

Table I.

Instar	Observed widths (mm.)	Average	Growth ratio	Width given by Sprengel (mm.)
1st	0.30, 0.30, 0.30, 0.32, 0.32, 0.32, 0.32, 0.35, 0.29, 0.29	0.311	—	0.376
2nd	0.40, 0.42, 0.42, 0.44, 0.44, 0.44, 0.49, 0.49, 0.49, 0.51	0.454	1.46	0.546
3rd	0.64, 0.64, 0.61, 0.66, 0.66, 0.67, 0.67, 0.67, 0.69, 0.69	0.663	1.46	0.683
4th	0.83, 0.83, 0.84, 0.84, 0.84, 0.86, 0.86, 0.86, 0.86, 0.86	0.848	1.28	0.915
5th	0.99, 1.01, 1.01, 1.03, 1.03, 1.03, 1.09, 1.09, 1.09, 1.29	1.066	1.25	1.051

V. SUMMARY.

H. flava L., which now appears to be the species definitely associated with injury to the developing fruits of the plum, is shown to be generally distributed over England and likely to be of economic importance where plums and damsons are grown extensively.

The adults are described and the chief points of difference between *H. flava* and other British Hoplocampids are mentioned.

The egg is described and figured and a full description of the larva and its various instars is given. The incubation period and larval development are discussed.

The writers are indebted to Mr F. R. Petherbridge, M.A., of the School of Agriculture, Cambridge, for consultation from time to time and for his keen interest in the progress of the investigations.

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EXPLANATION OF PLATES XXXIII AND XXXIV.

PLATE XXXIII.

Fig. 1. *H. flava* adults, ♂ (left), ♀ (right). × 5.

Fig. 2. Ovipositor (saws) of *H. flava*. × 120.

PLATE XXXIV.

Fig. 3. Egg of *H. flava* in calyx of plum blossom that has been broken open to show site of the egg. × 8.

Fig. 4. Larvae of *H. flava*. × 7.

(Received March 28th, 1933.)



Fig. 1.



Fig. 2.

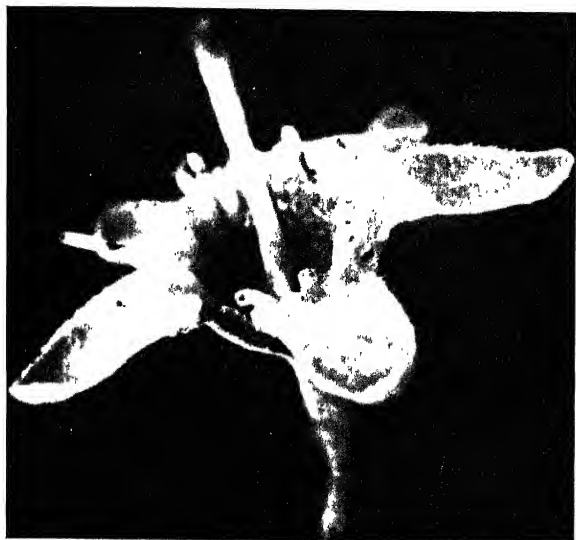


Fig. 3.



Fig. 4.

OBSERVATIONS ON THE FLIGHT MUSCLES OF *SITONA* WEEVILS

BY DOROTHY J. JACKSON.

(With Plates XXXV and XXXVI and 4 Text-figures.)

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INTRODUCTION.

In a previous paper (1928) a description was given of the macropterous and brachypterous forms of *Sitona hispidula* F. The breeding experiments conducted to determine the genetical relationship of the two forms were described, the results indicating that the brachypterous condition behaved as a simple Mendelian dominant. Attention was drawn to the

marked variation exhibited by the macropterous weevils in regard to the development of the fibrous muscles of flight: in some insects, regardless of sex, these muscles were fully developed, while in others they were greatly reduced and of abnormal histology. The weevils with well-developed wing muscles were capable of flight, but those with abnormal muscles had never been seen to fly. No differences were detected in the wings of the two forms, but it was noticed that the abnormal condition of the wing muscles was associated with slight modifications in the structure of the metathorax, certain parts normally serving for the attachment of the muscles of flight being reduced or absent. The most noticeable of these modifications related to the prephragma and the postphragma, those chitinous apodemes which, in the normal insect, project inwards from the anterior and posterior edge of the metatergum, and to which the greater portion of the median metathoracic muscle of flight is attached. In the weevils with abnormal wing muscles these apodemes were always greatly suppressed or entirely absent, while in weevils with normal wing muscles these apodemes were fully formed.

It was found subsequently that in some insects in which the chitinous supports of the muscles were fully formed the muscles of flight were not in a perfect state of development. Such specimens were only met with in the spring after hibernation, and the histology of the muscles of flight of these insects differed entirely from that of the abnormal muscles already referred to, and showed clear evidence of degeneration. Moreover a weevil which had been seen to fly in the autumn was found to have the muscles of flight in this degenerate condition when dissected the following April. This suggested that, in the normal insects, the capacity to fly might be restricted to a certain period in the life of the adult, after which the muscles of flight underwent degeneration. In the abnormal insects, on the other hand, my observations indicated that the power to fly was never attained, the muscles of flight remaining in an abnormal condition throughout the life of the weevil.

In order to investigate the problem of the variable condition of the muscles of flight in *S. hispidula*, it was necessary to examine the flight musculature at different periods of the adult life, both in the form capable of flight and in the form believed to be permanently flightless. Investigations were accordingly started in the spring of 1928 and continued over 2 years, as the life of the weevil in the adult condition alone, occupies fully a year. A study was also made of the post-metamorphic development of the muscles of flight in *S. lineata*, a species which is an excellent flier, and from the results of this research, combined with similar observations

on *S. hispidula*, valuable evidence was obtained regarding the relationship of the normal to the flightless form. Observations were also recorded on the proportion of normal to flightless insects obtained by breeding from macropterous weevils collected in the field, and some interesting data were thus acquired regarding the seasonal appearance of the two forms.

From the résumé of the literature which follows it will be seen that the occurrence of abnormal muscles of flight in winged insects has already aroused the interest of many entomologists, and it is hoped that the present work may contribute towards the elucidation of a difficult subject. It must be remembered that both *S. hispidula* and *S. lineata* are serious pests of leguminous crops in many countries, and the following information regarding their capacity for flight may also be of significance to the economic entomologist.

ON THE OCCURRENCE OF ABNORMAL MUSCLES OF FLIGHT IN OTHER
FULLY WINGED INSECTS, AND ON THE DEGENERATION OF NORMALLY
DEVELOPED MUSCLES AFTER FLIGHT HAS BEEN ABANDONED.

In several orders of insects cases have been recorded in which insects with fully developed wings are unable to fly owing to the abnormal condition of the muscles of flight, and in several species variation has been observed in the condition of development of the flight musculature in different individuals.

In the Forficulidae, Mercier and Poisson (1923) have investigated the muscles of flight in *Labia minor* and *Forficula auricularia*, species which have well-developed wings. *L. minor* flies well, and sections of the thorax showed voluminous muscular masses, some longitudinal and others dorso-ventral. In *F. auricularia*, however, the longitudinal muscles were considerably reduced and no trace was found of the dorso-ventral muscles, their position being occupied by sheets of fat tissue. Recently convincing evidence of the nocturnal flight of *F. auricularia* has been brought forward by Prof. Poulton (1930-2) in reference to the conclusions of Malloch that the common earwig was incapable of flight, Malloch having stated that the thorax of English species only contained traces of flight muscles. From these different observations there seems no doubt that in *F. auricularia* the flight muscles must either vary in development in different individuals or else undergo degeneration at some period of the adult life.

In the aquatic Hemiptera interesting cases are known in which the muscles of flight in certain fully winged insects are replaced by curious whitish organs, consisting of parenchymatous tissue, abundantly supplied

by tracheae. These tracheo-parenchymatous organs have been studied by various writers, especially by Ferrière (1914) and Poisson (1921, 1922, 1924), and they have been observed in *Nepa*, *Ranatra* and *Naucoris*. Ferrière considers that these organs have no function in themselves but are only transformed longitudinal muscles in process of atrophying, and in which the tracheae are preserved as they were in the normal muscle. The structure of the parenchymatous organs differs in the three genera both as regards the details of the tracheation and the character of the fibrous tissue. In certain of these aquatic Hemiptera the abnormal condition of the muscles of flight is not characteristic of all individuals of a species, in fact certain species show a dimorphism in regard to the condition of these muscles which is comparable to that occurring in *S. hispidula*. Thus in certain individuals of *Nepa cinerea* and *Ranatra linearis* the tracheo-parenchymatous organs may be replaced by perfectly developed thoracic muscles, but such individuals are less common than those with abnormal muscles. Ferrière observed that the insects with well-developed wing muscles were not limited to one sex, and they occurred both in the autumn and in the spring. In his dissections of *Nepa* and *Ranatra* made from October till May he never found transitional forms between the insects with muscular fibres and those with the transformed fibres, and he believes that at the end of the last moult certain individuals have normal muscles and others atrophied muscles. Poisson also draws attention to the absence of a continuous series of small graduated variations leading from normal muscle to abnormal muscle, and in *N. cinerea* he has observed that the muscular anomaly is hereditary. From the observations of both Ferrière and Poisson it is clear that the wings and elytra are always normally developed in the forms with abnormal vibratory muscles, and that there is no parallelism between the condition of the wings and that of the muscles of flight.

On the fly, *Chersodromia hirta*, Mercier (1920) has made some interesting observations regarding the flight musculature. Only certain individuals of this species were observed to fly, but in them the wings were no better developed than in those which did not fly. He found that the number of the fibres of the vibratory muscle varied in different individuals regardless of sex, and in a later paper (1926) he shows that this variation is customary in this genus.

In the Coleoptera, Rüschkamp (1927) points out that the presence of fully developed hind wings does not always coincide with capacity to fly, and he considers that none of the macropterous examples of the genus *Chrysomela* which he has studied are capable of flight. He has arrived at

this conclusion partly from observations in the field and partly by use of the so-called "Inselexperiments," in which the beetles to be tested are placed on an "island" and the food plant suspended out of reach. In addition he has made anatomical investigations of the flight musculature of some 250 *Melasma*, *Chrysomela* and *Chrysochloa*, but he gives little detail regarding the histology of the muscles of flight of these insects. Rüschkamp draws attention to the variation occurring in the condition of the muscles of flight during the life of the adult beetle, and he observed that in newly emerged beetles the flight musculature was not yet fully developed. He states that the muscles of flight undergo histolysis in the course of the imaginal life, but it is to be regretted that Dr Rüschkamp does not give more precise information on this subject. One misses any account of the histology of these muscles, either at their height of development or after degeneration has set in. His observations appear to be based entirely upon the examination of specimens collected in the field and not to have been corroborated by the study of bred specimens of known age.

Degeneration of the muscles of flight during the life of the adult is well known in certain insects which shed their wings after the period of flight is over. Thus in the ant queen, *Lasius niger*, the voluminous muscles of flight, which function only once for a few hours during an existence that may exceed 10 years, disappear completely shortly after the nuptial flight and become replaced by columns of adipocytes. The histolysis of the muscles of flight and the histogenesis of the fat body has been studied in great detail by Janet (1907a). He found that the histolysis of the vibratory muscles was not accompanied by phagocytosis, and he considers that the initial cause of histolysis resides in the muscle itself. Feytaud (1912) describes the degeneration of the muscles of flight in the termite imago after swarming. After the wings have been shed the muscles of flight disappear progressively and, at the end of some months, are completely replaced by fat tissue. He considers that the degeneration is accomplished by two methods, one by the fragmentation and simultaneous degeneration of the myoplasmic substance and the muscle nuclei, and the other by the development of phagocytes (derived, apparently, by proliferation from the nuclei of the muscle) which absorb droplets of the altered myoplasmic substance. Mercier (1924, 1928) describes a similar case of the atrophy of the muscles of flight in the flies, *Lipoptena cervi* and *Carnus hemapterus*. In these species, after the flies settle down to their ectoparasitic life, the wings break off and the flight muscles subsequently atrophy, being largely replaced by fat tissue.

COMPARISON OF THE LIFE HISTORY OF *SITONA HISPIDULA*
WITH THAT OF *S. LINEATA*.

The present investigations on the flight musculature of *S. hispidula* and *S. lineata* are closely concerned with the bionomics of these insects. It will therefore be advisable to give a brief summary of the main features of the life history of each species, as it occurs in Britain, for the benefit of those not already familiar with the habits of these insects.

The adults of *Sitona* feed upon the foliage, and the larvae upon the roots, of various leguminous plants. The adults of *S. lineata* are principally to be found during spring and summer on peas, beans and tares, but when these crops are harvested, they migrate to clover and lucerne, on which a certain number remain throughout the year. The adults of *S. hispidula*, on the other hand, feed principally on clover and lucerne and more rarely on other Leguminosae. Both species emerge from the pupal condition from July to September; during the early part of that period in the south of England and later in the north of Scotland. With *S. hispidula* oviposition begins in the autumn in Great Britain, but late-emerging British specimens do not breed till spring¹. With *S. lineata* sexual maturity is not attained till spring, when the majority of the weevils have migrated to young crops of peas and beans. The eggs of *S. lineata* hatch in about 3 weeks, those of *S. hispidula* take a few days longer, but the autumn-laid eggs of this species do not hatch till the spring. The larvae feed up during the summer, being most common during June and July. The duration of the larval period is usually longer in the breeding cages than under natural conditions, and in *S. hispidula* development is slower than with *S. lineata*. The pupal stage with *S. lineata* usually lasts 16–19 days, that of *S. hispidula* about 4 weeks. With both species there is only one generation in the year. The adults of *S. lineata* live only a year under natural conditions, but with *S. hispidula* the length of the adult life is more difficult to determine. In old weevils of *S. lineata* the scales become more or less rubbed off and, in the early summer, such dark-looking individuals are always to be found and can readily be distinguished from newly emerged specimens. With *S. hispidula* I have not found that the majority of the specimens collected at any one time of the year are abraded, though occasional worn specimens may be seen. It is

¹ In America, according to Wildermuth (1910), oviposition commences in the spring, but Bigger (1930) states that in Central Illinois it begins about the middle of October. My observations on Canadian weevils of *S. hispidula* brought to this country in August showed that only very few females were ovipositing in autumn, many eggs being laid in spring.

possible that the upstanding bristles so characteristic of this species afford some protection to the scales, and that the natural life of this weevil extends for more than a year, since at the time of writing some adults bred in captivity are still living, 2 years and 6 months after emergence.

S. lineata is, in the adult stage, a more active species than *S. hispidula*. Weevils of *S. lineata* have been found to fly freely both in autumn and spring when exposed to sunlight, but with *S. hispidula*, even those individuals able to fly have only been seen to do so in the autumn. It will be evident from the foregoing account that capacity for flight must be a valuable asset to *S. lineata* in its migrations from clover and lucerne to peas and beans and *vice versa*. With *S. hispidula*, on the other hand, ability to fly cannot be a matter of such importance, since clover, its principal food plant, is present either wild or cultivated in all but the most exposed localities. This species has been observed to be most active in the early autumn, and it is probably chiefly at this time that it reaches the new fields of clover, either by flight, or, where this is impossible, by crawling. The ease with which a flightless species can become dispersed throughout the clover in a field of first-year "seeds" is well shown in the case of *S. sulcifrons*, a species which, in Britain, is usually brachypterous. Numerous individuals of this species from the Scottish Highlands have been examined by the writer, and all have been short-winged, yet such flightless individuals are the most abundant species of *Sitona* amongst fields of young clover in the north of Scotland.

MATERIAL AND METHODS.

For the study of the muscles of flight in their normal and abnormal form, observations were made both on specimens of *S. hispidula* collected in the field and on those reared in captivity. The majority of the British specimens were collected at Harpenden, and many were obtained alive from Canada. Acknowledgments are due to Dr H. F. Barnes, Mr A. Gibson of Ottawa and Mr H. F. Hudson of Strathroy, Ontario, for help in procuring the weevils, and I am indebted to Prof. A. D. Imms for advice and criticism.

The rearing of specimens of the normal and of the abnormal forms of *S. hispidula* was beset with the obvious difficulty that one cannot tell whether any given insect reared from wild parents is destined to be of the normal or abnormal variety. Another species, *S. lineata*, which, with very rare exceptions, is an excellent flier, was therefore used for studying the post-metamorphic development of the normal muscles of flight. The

study of the abnormal muscles of flight was based principally on the examination of macropterous weevils of *S. hispidula* which had been bred in captivity for more than one generation, since it had been observed that the majority of such insects were of the abnormal form.

The weevils used for these investigations were reared at St Andrews, Fife, in the breeding boxes described in a previous paper (1928). The larvae of *S. hispidula* were reared on clover, grown in prepared soil according to the method already described (1928), and those of *S. lineata* were reared on beans, grown in ordinary garden soil, since no study was being made of the inheritance of the condition of the wing muscles in the latter species. The imagines of both species emerged during August and September. A careful search of the breeding cages was made daily, and the weevils found were placed in labelled tumblers in the insectary. Clover leaves were supplied in small bottles of water, and the mouth of the tumbler was covered securely with muslin. The weevils were then dissected at various dates after emergence.

When required for dissection the weevil was killed by exposure to chloroform vapour, the elytra were removed, the prothorax severed from the mesothorax and metathorax, and the abdomen ruptured, these incisions allowing for the penetration of the fixative, into which the specimen was then plunged. Various fixatives were used, such as picric alcohol, according to the directions given by Janet (1907 a); Bouin's picro-formol; Carnoy's acetic acid and chloroform; formalin; Flemming's solution with or without acetic acid, and most frequently the solution (No. 3) of sublimate acetic acid recommended by Tower (1903). This solution, which always gave most satisfactory results, consists of 60 parts of saturated solution of mercuric chloride in 35 per cent. alcohol, 10 parts of glacial acetic acid and 30 parts of a 2 per cent. solution of platonic chloride in distilled water. In some cases whole mounts were made of the abnormal muscles of flight or of teased-up portions of the normal muscles, but such material was usually too thick to give satisfactory results and the muscles to be studied were generally sectioned, either separate or attached to the metatergum. Longitudinal sections were most satisfactory for study of the histology. The material was often double embedded in celloidin and paraffin and sections were cut from 5 to 6 μ . The stains used were principally Heidenhain's iron haematoxylin, but Mayer's haemalum, as advised by Janet, was found very satisfactory for showing up the nuclei, and Biebrich scarlet or eosin was used as a counter stain.

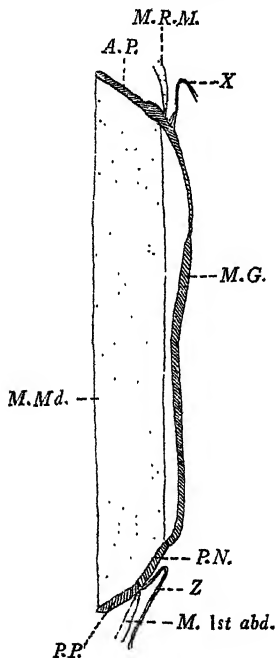
COMPARISON OF THE NORMAL AND THE FLIGHTLESS FORM
OF MACROPTEROUS *S. HISPIDULA*.

In the following account a description will be given of the structural differences in the metatergum and muscle discs of the normal and the flightless form, and the histology of the normal and of the abnormal muscles will then be dealt with.

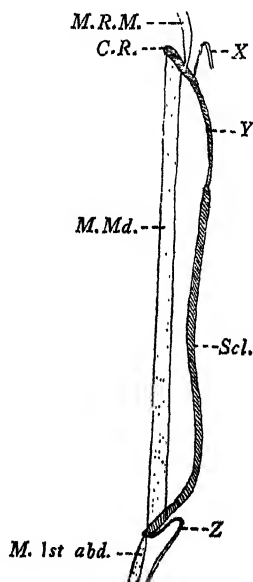
Structural differences in the metatergum and muscle discs.

The metatergum. Brief mention has already been made of the differences occurring in the structure of the metatergum in the two forms of macropterous insects. These relate principally to the degree of development of the prephragma and the postphragma, the apodemes which serve as the principal attachment of the large *musculus medianus metathoracis* in the insects capable of flight. In such forms the prephragma (Plate XXXV, fig. 1, *A.P.*) consists of two semicircular chitinous lobes which are attached to the prescutum, the anterior sclerite of the notum. They are situated between the lateral processes of the prescutum and project downwards into the body of the insect. At their base is a transverse chitinous ridge, immediately behind which, on the dorsal surface, the *musculus retractor mesonoti* arises. In the forms with abnormal wing muscles the prephragmal lobes are almost entirely absent, the region lying in front of the transverse chitinous ridge being narrow and almost straight in outline, and the narrow bundles of the median metathoracic muscle are attached to the transverse chitinous ridge at the anterior edge of the prescutum (Plate XXXV, fig. 2). The postphragma in the insects capable of flight (Plate XXXV, fig. 3, *P.P.*) consists of a transverse chitinous band attached to the postnotum, the posterior division of the metatergum. The postphragma is continuous with the posterior edge of the postnotum but slopes downwards beneath the first abdominal tergite. It does not extend along the whole length of the postnotum but is widest on each side of the median line and thence narrows towards the sides. The posterior limit of the postnotum is usually defined by a narrow band, more strongly chitinised. The thin membrane constituting the anterior portion of the first abdominal tergite is usually to be found reflected over the posterior part of the postnotum, so much so that in some specimens this membrane has the appearance of arising from the middle, or even from the anterior margin of the postnotum, but in longitudinal sections of the metatergum it is seen to be attached to the posterior edge of the postnotum just where the postphragma arises. The ends of the postnotum are expanded and produced into two points, and the expanded lateral area serves for the attachment

of the *musculus lateralis metathoracis tertius*. In the form with abnormal muscles of flight the postphragma (Plate XXXV, fig. 4) is almost entirely suppressed and the expanded lateral area of the postnotum is narrower.



Text-fig. 1.



Text-fig. 2.

Text-figs. 1, 2. Diagrammatic longitudinal sections of metaterga of *S. hispidula*, cut to right of median line, with a fibre of the median metathoracic flight muscle *in situ*.

Fig. 1. The normal form in which the flight muscles and their chitinous supports are perfectly developed, showing the prephragma and postphragma fully formed.

Fig. 2. The abnormal form in which the flight muscles and their chitinous supports are undeveloped. Note absence of prephragma and postphragma.

A.P. anterior phragma or prephragma; C.R. transverse chitinous ridge of prescutum; M.G. median groove of notum, right edge; M. 1st abd. longitudinal tergal muscle of first abdominal segment; M.M.d. fibre of *musculus medianus metathoracis*; M.R.M. *musculus retractor mesonoti*; P.N. postnotum; P.P. posterior phragma or postphragma; Scl. scutellum; X, intersegmental membrane connecting with mesotergum; Y, membranous area of prescutum; Z, tergite of first abdominal segment.

The posterior end of the median metathoracic muscle is attached in such insects to the postnotum. The relative position of a fibre of the median metathoracic muscle in regard to the metatergum in the two forms is shown in Text-figs. 1 and 2.

The relationship of the median metathoracic flight muscles to the prephragma and the postphragma has been studied in more detail in *S. lineata* by means of longitudinal vertical sections of the metatergum. It has been found that in the mature insect with perfect flight muscles, the hypodermal cells surrounding the apex of the prephragma and postphragma are large and with well-defined cell boundaries. The hypodermal cells of the postphragma of such an insect are figured in Text-fig. 4. In the rare flightless form of *S. lineata* (which is in every way comparable to the flightless form of *S. hispidula*) the hypodermis surrounding the anterior edge of the prescutum and the posterior part of the postnotum (where in the normal insect the phragmata would occur) consists of small flattened cells less than a quarter the size of those of the normal insect and with indistinct boundaries.

In the above account the terminology adopted is that used by Snodgrass (1909). In this paper he states (pp. 523-4) that the postphragma is really a chitinisation of the infolded intersegmental membrane behind the pseudonotum (postnotum) and that it is composed of two closely adpressed or fused laminae. I have not found this to be the case in the metathorax of *Sitona*, nor would the post-metamorphic development of this phragma (which will be described later) be easy to reconcile with this view. The postphragma appears merely to be a down-growth from the junction of the postnotum with the first abdominal tergite.

The muscle discs. The discs of the fibrous muscles of flight differ in size in the normal and the flightless insects. Thus the pronator disc is distinctly larger and of a deeper colour in the form with perfect wing muscles, and the disc of the *musculus lateralis metathoracis secundus* of the normal form is almost twice as large as in the abnormal form. Although the pronator disc is smaller in the abnormal insects, the slender muscles arising from it do not occupy all the surface of the disc, which is to be found largely filled with fat body.

*Structure of the flight muscles in the normal and in the
flightless form.*

The normal flight muscles. (Plate XXXVI, figs. 1, 3.)

General appearance. In the following account only the fibrous muscles of flight will be dealt with, and the small muscles of the tubular type occurring near the base of the wing will not be described. These tubular muscles appear to function in the folding of the wing, and they are normally developed even in those specimens in which the fibrous muscles are abnormal. This is what one would expect considering the fact that the

wings of such flightless weevils are always perfectly folded beneath the elytra.

In a weevil of *Sitona*, capable of flight, the fibrous muscles of flight occupy the greater part of the metathorax. In the freshly dissected insect they are of a translucent pale yellowish-grey colour but they become of an opaque white colour after fixation. In the fresh state the fibrous muscles have a uniform appearance owing to the great size and close juxtaposition of adjacent fibres, thereby they contrast with the muscles of the tubular type in which the fibres are relatively much smaller and more numerous. Since the topography of the muscles of flight has been described in an earlier paper (1928) it will be unnecessary to give an account of it here. The indirect wing muscles (which, according to their position, cause the arching or the flattening of the tergum and thus bring about the lowering or raising of the wings) include the *musculus medianus metathoracis*, the prominent longitudinal dorsal muscle; the *musculus lateralis metathoracis tertius*, consisting of two bundles on each side which run obliquely from just behind the anterior apodeme of the main scutal lobe to the lateral expanded area of the postnotum; and the *musculus lateralis metathoracis primus* and the *musculus lateralis secundus* which run obliquely from the tergum to the sternum. The direct wing muscles include the tubular muscles already referred to, and the large *musculus extensor alae* (a muscle of the fibrous type) at the side of the metathorax which is inserted in the pronator muscle disc of the pleurum.

Histology. The histology of the normal fibrous muscles of flight in *Sitona* presents no peculiarities, but will be described briefly to allow of comparison with the histology of the abnormal muscle. In fixed muscle preserved in alcohol the individual bundles composing each muscle can be readily separated. Repeated dissections and the examination of numerous sections have convinced me that each bundle must be looked upon as a single large fibre. As compared with the size of the fibres of ordinary tubular muscle, the fibres of the fibrous muscle are enormous. The fibres vary in size and are not always of uniform width throughout. Thus the six fibres which compose the median metathoracic muscle differ in size and are narrow anteriorly and widen posteriorly. In fixed preparations the largest fibres of this muscle have measured 110μ anteriorly and 160μ posteriorly. The largest fibre of the fibrous muscles is the single bundle of the *musculus lateralis secundus* which may attain a width of 240μ . When this bundle is examined in the fresh state its surface has the appearance of being divided longitudinally into parallel columns between which the tracheae penetrate, and it was thought that these columns might repre-

sent separate fibres. But sections of the bundles showed that the "columns" were merely peripheral lobes formed by clefts which penetrate a short distance into the muscle bundle, but do not extend to its centre. This bundle must therefore be considered as a single giant fibre.

Each fibre has a smooth outer surface, being surrounded by a membrane which shows distinctly in both transverse and longitudinal sections. The existence of a membrane surrounding the fibre in insect muscles of the fibrous type has been observed by some writers: Janet (1907 *a*), Poisson (1924), Athanasiu and Dragoiu (1915), but is denied by others: Berlese (1909), Henneguy (1904), Morison (1928), Breed (1903). In the beetle, *Thymalus marginicollis*, Breed (1903) could not demonstrate a sarcolemma for the individual fibres of the wing muscles, but Kielich (1918) in his description of the wing muscles of *Hydrophilus piceus* considers that the smooth contour of the muscle bundle denotes the presence of such a membrane. Athanasiu and Dragoiu (1915) consider that the muscular fibres of the wings have not a true sarcolemma, but that each fibre is surrounded by a mass of mesenchymatous cells which form a veritable envelope.

The fibres of the fibrous muscles of *Sitona* can be readily split up, either in the fresh or fixed condition, so as to disclose their multitude of constituent fibrils or sarcostyles. The width of the sarcostyle varies according to whether it is examined fresh or fixed, contracted or in a resting condition. In fresh muscle (of both *S. lineata* and *S. hispidula*) examined in normal salt solution (0.75 per cent.) the fibrils measure from 3μ to just over 4μ in diameter, in fixed preparations they measure usually about 2μ , sometimes a little more or less. The cross-striation of the fibrils is most distinct in fresh muscle, split up and examined in salt solution.

The nuclei in mature fibrous muscle occur in long parallel rows which extend usually throughout the length of the muscle fibre. The arrangement of the nuclei is best seen in fresh wing muscle examined in normal salt solution, after splitting up the fibre and staining with methylene blue. In the unstained fresh muscle the nuclei cannot be distinguished, but they readily stain in methylene blue. In such preparations they can be examined unshrunk and they measure usually about 8μ by 3μ . In fixed preparations, sectioned, their average size is less, about 6μ . The nuclei in each row are usually situated close together, sometimes almost touching each other, or with a space between equal to, or slightly greater than, the length of a nucleus. In places, however, a short row is to be seen consisting of larger and more elongated nuclei, 8μ to 12μ in fixed preparation, up to 16μ in fresh muscle, and these nuclei are much more widely

separated from each other. It is probable that when many nuclei are present in one row the adjacent fibrils are pushed farther apart, but the more solitary nuclei will tend to be more compressed from side to side. In longitudinal sections the rows of nuclei are seen to lie from 5μ to 12μ apart and three or four fibrils occur between the rows.

The fibrils and nuclei are embedded in the sarcoplasm which, in suitably fixed preparations, appears to consist almost entirely of masses of sarcosomes (Plate XXXVI, fig. 3). The sarcosomes show up distinctly after fixation in a 10 per cent. solution of formol, and they are also distinguishable in preparations fixed in Tower's sublimate acetic acid. They are small disc-like bodies of rounded or irregular shape, which vary in size from 1.2μ by 0.8μ to 2.5μ by 1μ . In some preparations, where the fibrils lie close together, the sarcosomes show a fairly regular arrangement; a single row may occur between two adjacent fibrils, the sarcosomes of the different rows coinciding in position, one to each sarcomere, so that the appearance of cross-striation of the muscle fibres is enhanced. In other sections, where the fibrils are more widely separated, the sarcosomes are crowded irregularly into the spaces between them. Such irregularity of arrangement may well be an artefact due to distortion of the fibre during dissection of the thorax.

The muscles of flight of *Sitona* are abundantly supplied with tracheal tubes. In sections of the muscle the finer tracheae, measuring from 3μ to 4μ in diameter and showing distinct taenidia, may be seen penetrating the peripheral membrane of the fibre and passing between the fibrils. Such tracheae subdivide and from the ends of the finer branches, measuring 1.25μ – 2μ in diameter, the tracheoles arise; a considerable number originate from each tracheal branch, the end of which, for a distance of not more than 9μ , is devoid of taenidia. The tracheoles are long and extremely slender tubes, and it was judged that the breadth of three would be equivalent to a space 1μ wide. They extend for a considerable distance at a uniform thickness, but are a trifle wider at their point of origin, and occasionally a tracheole slightly thicker than the rest may show a bifurcation near its base. A single nucleus, usually larger and more elongated than those on the tracheae and often truncated at the distal end, occurs amongst each group of tracheoles near their point of origin. This nucleus is apparently supported by one of the larger tracheoles, and it may be situated close to the base of the tracheole or up to 15μ distant from the base. Farther along the tracheoles no nuclei could be detected. The tracheoles show up very distinctly in fresh wing muscle examined in dilute glycerine, but once the air is expelled from them they become

difficult to see. They penetrate in all directions in the muscle, but are not arranged with any regularity in regard to the fibrils, in fact comparatively large areas of fibrils are quite unsupplied with tracheoles. This is very different from the regular arrangement of tracheal capillaries described by Athanasiu and Dragoiu (1915) in the muscles of flight of the beetles, *Hydrophilus piceus* and *Lucanus cervus*. These writers, from their study of silver impregnations of these muscles, concluded that each fibril is in intimate association with tracheal capillaries throughout its length, in fact that each dark disc of the fibril is related at each of its four lateral faces to a tracheal capillary. In *Sitona* the tracheoles (which are even smaller in diameter than those described by Athanasiu and Dragoiu) are not present in sufficient numbers to allow of such close association with each fibril, and even in fibres extracted unruptured from a weevil, no such regularity of arrangement of the tracheoles can be observed.

The abnormal flight muscles. (Plate XXXVI, figs. 2, 4.)

General appearance. The abnormal muscles of flight have an entirely different appearance from the normally developed fibrous muscles. In the freshly dissected insect they form narrow whitish bands, sometimes so thin as to be semi-transparent, and they are largely surrounded by fat body, the fibres of the median metathoracic muscle being frequently enclosed in a web-like mass of fat tissue. The width of each bundle or fibre is only half or even a third of that of the normal muscle; thus the median metathoracic bundles rarely exceed 50μ in width in the middle (the expanded posterior end is slightly wider) and the *lateralis secundus* which normally attains a width of nearly 240μ is only about 60μ wide in the flightless insects.

Histology. The detailed structure of the abnormal muscles is characterised by the absence of fibrils and the abundance of the nuclei relative to the size of the fibres. The nuclei are not arranged in regular rows as in normal muscle, but are thickly scattered throughout the fibre. There is an abundance of long nuclear masses measuring from 20μ to 40μ in length and consisting of numerous segments in process of division; the newly separated segments are short (about 5μ) and bluntly truncated. The width of the nuclear masses is usually that of a single nucleus, but some are wider, and in such, a double row of segments can be distinguished in the centre. Between the long nuclear masses occur solitary nuclei of normal shape measuring usually about 10μ long, but occasionally much larger nuclei are to be seen which may attain a size of 24μ long by 9μ wide. While no fibrils can be distinguished in these muscles, the sarco-

plasm shows a longitudinal striation appearing as excessively fine lines about 0.2μ in thickness. The cause of this striation is difficult to ascertain with certainty, but I consider that it is principally, if not entirely, due to the numbers of parallel running tracheoles which traverse the fibre throughout its length. If the abnormal muscles are freshly dissected from a newly killed weevil and examined in a dilute solution of glycerine, they appear when viewed with low powers of the microscope as greyish bundles longitudinally fibrillated. Under higher magnification the longitudinal striae are seen to be fine parallel-running tracheoles which are so numerous that the muscle has the appearance of being composed of little else. The tracheoles arose (as in the normal muscle) in a bunch from each ultimate tracheal branch, and owing to the narrow width of the fibre they are forced to run up or down the fibre, closely parallel to each other. In normal fibrous muscle, on the other hand, the great width of the fibre permits the tracheoles to diverge in all directions.

The structure just described as typical of abnormal muscle has been observed in weevils of various ages in which the prephragma and postphragma were quite undeveloped. A careful comparison has been made, with the assistance of camera lucida sketches, of the flight muscles in abnormal insects, 1 day old¹, 7 weeks, 14 weeks, 8 months, 17 months and 18 months respectively, but no appreciable change has been observed in the histology of the muscles, except that in the newly emerged insect the nuclei are a little smaller and slenderer. Even in the 17-month-old weevil many of the nuclei are to be found in the form of long segmenting masses, but it is impossible to say whether this is due to a fresh division of the nuclei, or to the persistence of the original nuclear segments in their original conformation.

Intermediate forms. Forms intermediate between the normal and abnormal type have been observed both amongst weevils collected in the field and amongst those reared in captivity. In such specimens the phragmata and muscle discs of the metathorax are not fully formed and the muscles of flight may be larger than in typical abnormal insects. Thus the prephragma may be partially developed and the postphragma absent, or the prephragma may be fully formed and the postphragma diminutive. The histology of the flight musculature of such an insect is of intermediate character between the normal and abnormal type, and the same muscle fibre may show variation in development throughout its length, very fine fibrils, under 1μ in diameter, being distinguishable at the ends of such

¹ A specimen from Breeding Experiment A was used for this observation as all the offspring bred from this couple proved to be of the abnormal type.

a fibre and not in the centre. In these intermediate forms several instances of asymmetry have been observed in the development of the phragmata, and this may be correlated with a greater development of the flight muscles on the one side of the insect than on the other. A somewhat similar case of the asymmetrical development of the vibratory muscles is recorded by Mercier (1920) in the fly, *Chersodromia hirta* Walk.

POSITION OF THE TERMINAL CHAMBERS OF THE OVARIES IN
REGARD TO THE MUSCLES OF FLIGHT.

In newly emerged females of *S. hispidula* the ovarian tubules are so small and undeveloped that they are insignificant in bulk as compared with the fibrous muscles of flight. Later on, when the female becomes sexually mature, either in the autumn or in the spring, the ovarian tubules have increased enormously in size, attaining to more than twice their original length and breadth, and the muscles of flight now look diminutive beside them. In weevils in which the muscles of flight are fully developed, these muscles occupy the greater part of the metathorax, and in sexually mature insects only the apical portion of the terminal chambers of the ovaries projects into the metathorax. But in the insects with abnormal wing muscles much more space is available in the metathorax, and the terminal chambers of the ovaries lie immediately under the metatergum. In those weevils in which the normal muscles of flight undergo degeneration during the winter, the terminal chambers of the ovaries likewise spread into the metathorax.

These facts led one to consider whether there might be any correlation between the time of development of the reproductive organs and the condition of the muscles of flight, and whether the forms which became sexually mature early were those in which the fibrous muscles never attained normal development. Dissections were accordingly made of a number of bred specimens of *S. hispidula*, but the results clearly indicated that the condition of development of the ovarian tubules was quite unrelated to the condition of development of the flight muscles; in fact relatively more specimens with mature ovaries were to be found amongst the weevils with perfect flight muscles than amongst those in which the flight muscles and phragmata had never attained normal development.

DISTRIBUTION OF THE NORMAL AND THE FLIGHTLESS FORM
OF MACROPTEROUS *S. HISPIDULA*.

In collecting specimens of *S. hispidula* for a study of the distribution of the normal and the flightless form, a superficial examination of the condition of the flight muscles in the newly killed insects does not always suffice to discriminate between the two forms. If the muscles are found to be in a reduced condition this may merely be due to the degeneration which, as will be shown later, frequently occurs in the normally developed muscle of hibernated weevils. It is therefore necessary to examine the metatergum of each insect having defective flight muscles, since, if the weevil has been capable of flight, it will be found that the prephragma and postphragma are well formed. Newly emerged insects are unsuitable for this study, since, as will be described in the subsequent section, the flight musculature of such insects, even though destined to be of the normal kind, would not have attained its full development. With these restrictions the collecting of reliable data is a somewhat laborious task, and the list which follows is based on the examination of over 200 specimens from various localities in Britain and North America. These records show that the flightless macropterous weevils are widely distributed and that the proportion of normal to flightless insects varies in different localities. In the north of Scotland it would appear from the data available that there is a much greater proportion of flightless to normal forms than occurs farther south, and a possible reason in explanation of this will be discussed later.

Great Britain. Wye, Kent: undated, 1 normal. Haslemere, Surrey: April 1924, 4 normal. Harpenden, Herts.: 1927-30, 26 normal, 6 intermediate, 1 abnormal. Tring, Herts.: April 1921, 1 normal. Oxford: August 8th, 1926, 2 normal. Sudbury, Suffolk: 1929-30, 13 normal, 2 intermediate. Aberystwyth: 1927, from Mr J. R. W. Jenkins, 38 normal, 2 intermediate, 1 abnormal. St Andrews, Fife: undated, 2 normal, 1 abnormal. Kingussie, Inverness-shire: April 1921, 1 normal, 2 abnormal. Evanton, Ross-shire: 1921-6, 2 abnormal. Invershin, Sutherland: 1921-6, 1 normal, 3 intermediate, 14 abnormal.

America, U.S.A. Forest Grove, Oregon: from Mr L. P. Rockwood, 1914-16, 16 normal, 1 intermediate¹. Geneva, New York: August 15th, 1928, 2 normal. Ithaca, New York: August 1928, 1 normal.

¹ In my previous paper (1928) reference was made to several specimens from this locality having muscles of flight of reduced size, but since no examination was made of the metaterga of these insects it is not possible to state now whether the condition of the muscles was of the usual abnormal type, or merely normal muscle degenerated.

Canada. Strathroy, Ontario: August 15th, 1928, 59 normal, 3 intermediate, 8 abnormal.

ON THE POST-METAMORPHIC DEVELOPMENT OF THE MUSCLES OF
FLIGHT AND THEIR CHITINOUS SUPPORTS IN *S. LINEATA*.

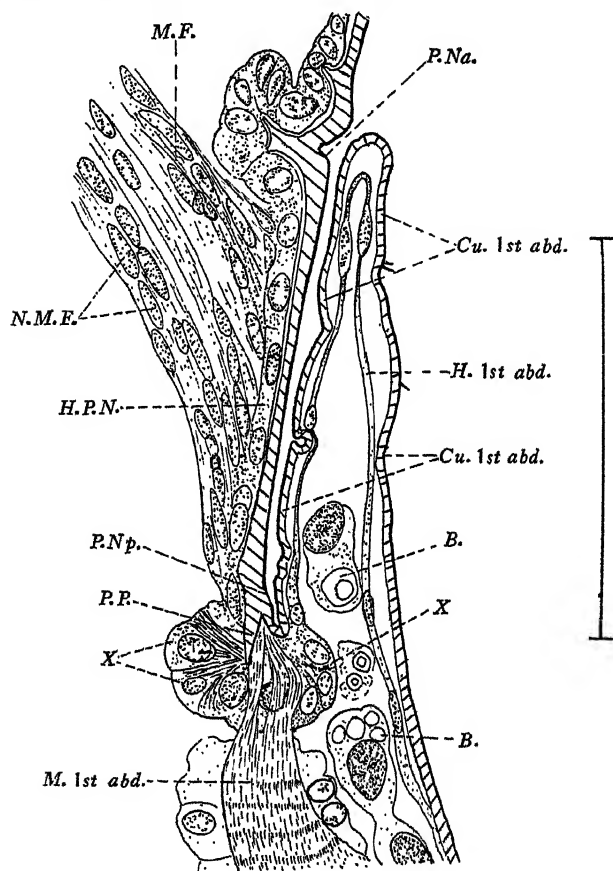
In weevils of *S. lineata* which have newly emerged from the pupal cells it was found that the muscles of flight and their chitinous supports were structurally different from those of the mature weevil, and resembled in many respects those of the flightless form of *S. hispidula* already described. The normal condition of the flight muscles and the metatergum was not attained until some time after emergence. In the following account the development of the chitinous supports of the muscles of flight will be dealt with first and the histology of the developing muscles will be described afterwards.

Development of the chitinous supports of the muscles of flight.

In a newly emerged weevil, the tergites of the metathorax and of all but the last two abdominal segments, are colourless and transparent. The cuticle of the metatergum is extremely thin, averaging 4μ thick, and the contents of the metathorax seen through it usually appear white in colour. This is due largely to the mass of minute bodies of excretory nature (Text-fig. 3, B.)¹ which are located in the fat of most newly emerged weevils of this species and on dissection are also to be found free in the haemocoel. These bodies obscure the narrow bundles of the median metathoracic muscles, which in newly emerged weevils of *S. hispidula* are clearly visible through the tergite. If the metatergum be dissected off and examined, it will be seen that the prephragma and the postphragma, the principal supports of the median metathoracic muscles of flight, are absent. The other supports of the fibrous muscles are present, but they are of considerably smaller size than in the adult insect. Thus the pronator disc is smaller and has the posterior part much less developed than in the mature insect, the expanded ends of the postnotum are shorter, and the disc of the *musculus lateralis secundus* is diminutive as compared with its final size. By dissecting weevils of different ages the gradual development of these chitinous parts has been followed. Thus in a weevil

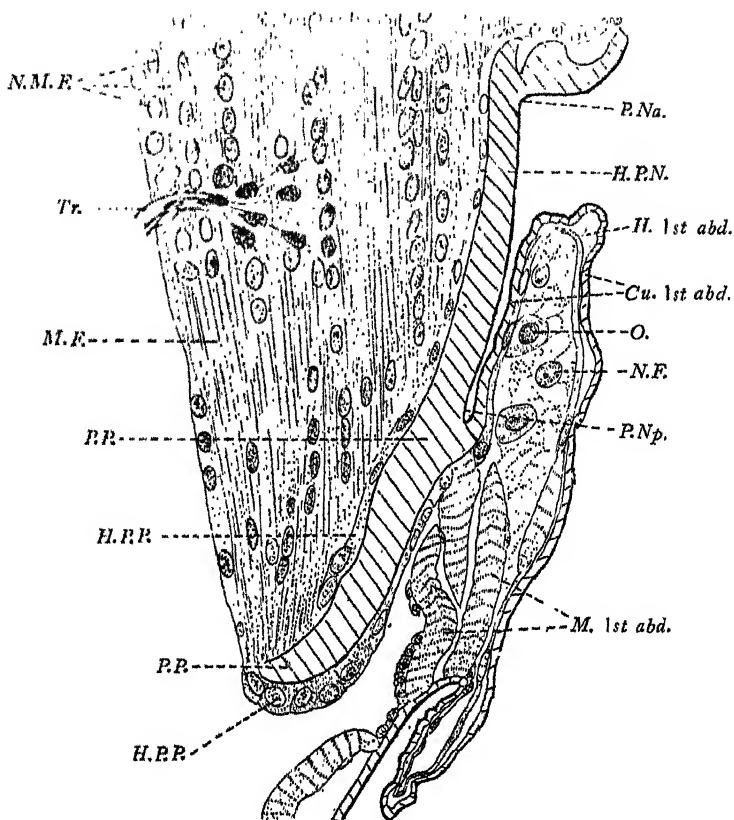
¹ These bodies, which vary in size from 3μ to 8μ , are round, oval, or irregular in shape, and doubly refractile in polarised light; they have been examined by Dr D. Keilin who states that they are of excretory origin, very probably urates or calcospherites. I have observed them also in the larva and pupa of *S. lineata*, but, in the adults, they have been found to occur only in the newly emerged insects. Strangely enough I have not as yet found these bodies in newly emerged weevils of *S. hispidula*.

killed 10 days after emergence, the metatergum had become dark brown in colour and the cuticle was thicker. Though the wing muscles were now more or less fully developed, the muscle discs had not attained their full size. The prephragma and the postphragma were only in process of development, appearing as thin colourless projections covering the



Text-fig. 3.

anterior and posterior ends of the median metathoracic muscles. These developing phragmata show up well if the metatergum is treated with caustic potash and then stained with Bleu de Lyon, since, being colourless, they stain strongly with the blue, contrasting with the dark brown colour of the remainder of the metatergum. In a weevil killed 18 days after emergence these phragmata were still in course of formation and pale in



Text-fig. 4.

Text-figs. 3, 4. Longitudinal vertical sections of postnotal region of metatergum from two weevils of *S. lineata*. Chitinous parts represented by oblique shading.

Fig. 3. From a weevil killed when newly emerged from pupal cell. The flight muscles and postphragma are still little developed, the postphragma being represented by a minute spur at the end of postnotum. $\times 937\frac{1}{2}$. Owing to the smaller size and more complex structure, this figure has had to be drawn to a higher magnification than Fig. 4, but the length of this section at same magnification as Fig. 4 is indicated by a line parallel to Fig. 3.

Fig. 4. From a weevil killed 26 days after emergence from pupal cell. The flight muscles and postphragma of this insect are well developed. The cross-striation of the flight muscles is now distinct but has been omitted from the figure in order to simplify the drawing. $\times 450$.

B. body of excretory nature located in fat cell; *Cu. 1st abd.* cuticle of first abdominal tergite; *H. 1st abd.* hypodermis of first abdominal tergite; *H.P.N.* hypodermis of postnotum; *H.P.P.* hypodermis of postphragma; *M.F.* median metathoracic muscle of flight; *M. 1st abd.* longitudinal tergal muscle of first abdominal segment; *N.F.* nucleus of fat cell; *N.M.F.* nuclei of muscle of flight; *O.* oocyte; *P.Na.* postnotum, anterior edge; *P.Np.* postnotum, posterior edge; *P.P.* postphragma; *Tr.* trachea; *X.* hypodermal cells surrounding developing postphragma.

colour; at 26 days they were further developed and faintly brown¹; at 6 weeks they were well developed but the tips of the prephragma still pale. In an 8-week-old weevil these phragmata were fully formed and had acquired their normal colour, which is darker than that of the adjoining parts of the metatergum. At this stage the other muscle discs had also attained their full size. The cuticle of the entire metatergum had increased to about three times the thickness of that of the newly emerged insect.

While the general development of the prephragma and postphragma can be readily observed by dissecting weevils of different ages and making microscopic mounts of the parts concerned, the detailed development of these parts is best understood from an examination of longitudinal vertical sections of the metatergum, since the varying thickness of the hypodermis can best be seen in such sections. In the newly emerged weevil the hypodermis of most parts of the metatergum forms a layer of flattened cells less than 1μ thick, the boundaries of which are most difficult to detect. In surface view the hypodermal nuclei are oval to round in shape, but in section they appear elongated, being flattened dorso-ventrally. At those parts of the metatergum where the cuticle is thicker, as at the sides of the median groove of the notum, at the muscle discs and around the internal apodemes, the hypodermis is much thicker, owing largely to the nuclei being more numerous; the cell boundaries are apparent and the cells may be of upright columnar shape. A great activity of the hypodermis occurs at the anterior edge of the prescutum, where later the prephragmal lobes will form, and at the posterior edge of the postnotum, where the postphragma will develop. It is generally believed that the cuticle is a product of the underlying hypodermis and therefore one would expect the hypodermis to be especially active at those parts where the cuticle or an apodeme is in process of rapid development.

The formation of the prephragma takes place as follows. In the newly emerged weevil the median metathoracic muscles of flight, which are still small and in immature condition are attached (as in the flightless form of *S. hispidula*) to the anterior edge of the prescutum just beneath the transverse chitinous ridge. This ridge lies beneath the intersegmental membrane connecting the metatergum with the mesotergum, and it is of course surrounded by a down-growth of the hypodermis. It is around the apex of this down-growth that the hypodermal cells show such a marked

¹ The weevil of 26 days, the postnotum of which is figured in Text-fig. 4, was reared during another season, and when killed, was in a more advanced condition than the specimen here referred to, having the postphragma (P.P.) well formed.

increase in size as compared with the undifferentiated hypodermis of other regions of the cuticle. During the period when the flight muscles are attaining their normal structure, further development of the hypodermal cells occurs at this point and more chitinous matter is deposited within this gradually enlarging hypodermal sac, until the prephragmal lobes are thereby formed. They now afford support for the greater portion of the fibres of the median metathoracic muscle which, at this time, is fully developed. The hypodermal cells are always largest nearest the apex of the prephragma, and more columnar in shape, thence they decrease in size basally and become flattened. In two weevils with fully formed flight muscles and phragmata, killed when over 6 weeks old, the hypodermal cells at the apex of the prephragma were even larger in size than in the newly emerged insect and they projected beyond the prephragma around the ventral base of the muscle. They measured in section as much as 20μ high or about three times the height of those of the newly emerged weevil.

The development of the postphragma occurs in much the same way as that of the prephragma. In longitudinal sections of a newly emerged weevil the postphragma is either entirely absent, the postnotum ending bluntly, or the postphragma is represented by a minute spur at the apex of the postnotum (Text-fig. 3, *P.P.*). The hypodermis of the postnotum is thicker than that of the undifferentiated parts of the tergum, measuring 6μ thick. At the posterior edge of the postnotum the hypodermal cells are greatly enlarged and bulge out around the part where the postphragma is about to develop (Text-fig. 3, *X*). In these cells, which measure 14μ – 18μ high, longitudinal thread-like strands are visible traversing the cell from periphery to base, and since these cells are arranged in a semicircle with their basal ends in direct contact with the minute spur which is the developing postphragma, these strands are all directed to it. Such threads have been recorded in hypodermal cells in process of chitin formation by Holmgren (1902), while Kühnelt (1929) and Casper (1913) describe a vertical striping in the upper part of hypodermal cells in the act of chitin formation. This short vertical striping in the part of the cell lying next the cuticle, shows in sections of the hypodermis surrounding the prephragma in a weevil of *S. lineata* killed 26 days after emergence. The longer strands traversing the cells surrounding the developing postphragma in the newly emerged weevil are probably a phenomenon associated with a rapid formation of chitin. In some of these cells drops of homogenous substance also occur which may be droplets of secreted chitin homologous with the vacuoles observed by Kapsov (1911)

in the hypodermal cells of the pupa of *Cetonia aurata*. These long threads and vacuoles have not been observed in the hypodermis of weevils in which the postphragma is fully formed, and chitin formation consequently at an end.

As the weevil becomes older the bulge of hypodermal cells around the apex of the postnotum widens out and grows downwards, the chitin of the postphragma being deposited within it. Thereafter, just as with the prephragma, the hypodermal cells of the basal region of the postphragma diminish in height and become flattened, but the cells at the extreme tip remain very large even although the postphragma is fully formed, and in a weevil over 6 weeks old these cells measured 20μ – 22μ high. The appearance of the postnotal region in sections of a weevil of 26 days is shown in Text-fig. 4. The homologies of the postnotal region in *Sitona* are a little complicated by the fact that the anterior portion of the first abdominal tergite is strongly reflexed over the greater part of the postnotum. The dorsal longitudinal muscles of the first abdominal segment are attached to the posterior extremity of the postnotum, the fibrils penetrating through the hypodermis and being inserted directly in the chitin. The median longitudinal muscles of flight are attached in the newly emerged weevil to the postnotum (Text-fig. 3, *M.F.*) but as the flight muscles and the postphragma develop coincidently (although the postphragma arrives at its full development a little later than the muscle), in the mature insect the greater part of the fibres of this muscle is inserted in the postphragma (Text-fig. 4, *M.F.*).

The hypodermis of most parts of the cuticle of the metatergum preserves its original character, and does not appear to degenerate in old beetles of *Sitona*, as has been recorded in *Dytiscus* by Casper (1913), since in an old weevil of *S. lineata* in which the flight muscles had degenerated the hypodermis was of normal appearance.

Development of the muscles of flight.

The direct wing muscles of the tubular type, the *relaxator alae* and the *relaxator extensoris*, are further developed in the newly emerged weevil than are the fibrous muscles. The individual fibres of these small tubular muscles are, however, narrower than those of the mature insect and cross-striation has not been detected in them, though the other tubular muscles of the metathorax, such as the *musculus retractor mesonoti*, are well developed at this stage and cross-striated.

The condition of the fibrous muscles of flight varies in newly emerged individuals and may even be variable in the same individual. In the

majority of newly emerged weevils examined, the fibrous muscles showed the following structure (Plate XXXVI, fig. 5). The bundles (or fibres) were extremely narrow as compared with those of an insect capable of flight; thus the bundles of the median metathoracic muscles varied in breadth from 30μ – 56μ in the centre, to 50μ – 80μ at the expanded ends. The general appearance of these immature muscles was closely like that of the abnormal muscles of flight already described, and their histology was also similar. The nuclei, which are crowded together in the fibres, are quite different in appearance from the nuclei of functional muscle. The majority of the nuclei are very long, and are evidently undergoing direct division throughout their length. Various stages may be seen between the long undivided nuclei (which may attain a length of 22μ), those in which segmentation is only commencing and those in which division has been completed, so that the newly divided segments form chains of small nuclei from 1.5μ to 3μ wide. Occasional large nuclei occur which are wider than the rest and of more oval shape and these have also been observed in abnormal muscle. In addition there are always present a number of smaller solitary nuclei measuring about 8μ long. The substance of the muscle between the closely placed nuclei is seen to be longitudinally striated. Careful observation has convinced me that at this stage no fibrils are developed, and that the longitudinal striae are entirely due (as in the abnormal muscle) to numbers of parallel-running tracheoles. An examination has been made in glycerine of fresh wing muscles from a newly emerged weevil, and under the high power each fibre appears as though composed of masses of longitudinally running tracheoles. While the muscles of flight of the newly emerged weevil were thus similar in many respects to the abnormal muscles of mature weevils, it was found that in the abnormal muscles the fibres were thicker and the nuclei were larger and less elongate.

A more advanced stage in the development of normal wing muscle than that just described was found in one newly emerged weevil. In this specimen, the muscles of flight, though still very narrow, showed fewer nuclei in process of division, and more newly formed nuclei, appearing as separated segments with truncated ends, while many other nuclei had assumed an elongate oval shape. Tracheoles were greatly in evidence in sections of these muscles, the large nucleus occurring at the base of each group of tracheoles being very prominent and staining darkly. In this insect one of the fibres of the median metathoracic muscles showed a much more advanced condition of development than any of the others, being more than three times greater in width. In this fibre fibrils were

present, cross-striation was discernible and the nuclei occurred in even rows; in fact the histology of this fibre approached that of mature muscle, but the nuclei were larger, more variable in size and less elongated in shape. Stages intermediate between this muscle fibre and the others were not observed in *S. lineata*, but in *S. hispidula* it was found that in fibres of intermediate development the fibrils were much slenderer (under 1μ in diameter) than in mature muscle, and exhibited no cross-striation.

In a weevil of *S. lineata*, killed 10 days after emergence, the wing muscles were found to be large and fully developed, filling up the metatergum, although the muscle discs had not attained their full size. Histologically these muscles showed a later stage of development than in the advanced fibre of the newly emerged insect described above, the nuclei being of more regular size and the fibrils thicker.

The attachment of the median longitudinal muscles of flight to their chitinous supports can best be seen in longitudinal vertical sections of the metatergum. In the mature insect with perfect flight muscles, the tonofibrillae into which the fibrils resolve themselves may be seen penetrating in groups between the nuclei of the hypodermal cells to insert themselves in the chitin. In the newly emerged weevils no tonofibrillae have been observed in the hypodermal cells at the point where the immature muscles are inserted, and it is probable that the development of the tonofibrillae is coincident with that of the fibrils.

Since the rate of development of the muscles of flight varies in different individuals, it is impossible to state with any precision the time taken by these muscles to become fully mature. It was noticed, however, that in younger flight muscles the nuclei were more abundant relatively to the fibres than in older muscles, indicating that the fibrils increase in number as the muscle matures. Thus in a weevil killed 6 weeks after emergence only half as many nuclei were to be counted in a given area of a fibre, as in an area of the same size in a weevil killed at 26 days.

While in the majority of weevils of *S. lineata* the muscles of flight were found to be fully developed in less than a month after emergence from the pupal condition, in some individuals which emerged late in the autumn of 1928, and in others bred during the wet summer of 1931, the normal condition was not attained, and in such weevils the condition of the flight muscles and their chitinous supports was identical with that of the flightless individuals of *S. hispidula* already described. In the field such flightless individuals of *S. lineata* are rare and have only been met with once (see p. 766) although large numbers of weevils from many localities have been collected and dissected.

ON THE POST-METAMORPHIC DEVELOPMENT OF THE MUSCLES OF
FLIGHT AND THEIR CHITINOUS SUPPORTS IN *S. hispidula*.

In newly emerged weevils of *S. hispidula* the condition of the metatergum, the muscles of flight and their chitinous discs was found to be just the same as in newly emerged weevils of *S. lineata*. In *S. hispidula*, however, owing to the absence of excretory granules, the median metathoracic muscles can be seen through the metatergum of the newly emerged insect, appearing as narrow whitish bands. This fact allowed me to observe the growth of the muscles of flight in a single individual, for, by raising the elytra of a living newly emerged weevil, the muscles were seen to be in their usual undeveloped state, and on ~~killing the same insect~~ 11 weeks later the muscles were then found to be fully developed.

The outstanding point of difference in regard to the post-metamorphic development of the muscles of flight in this species as compared with *S. lineata* was that only in certain individuals of *S. hispidula* did this further development take place. In many individuals the muscles of flight and their chitinous supports did not develop (except in minor details) beyond the condition characteristic of the newly emerged insect, though the cuticle of the metatergum attained its normal thickness and pigmentation. Such individuals are the ones hitherto described as having abnormal muscles of flight but which can now more truly be defined as having the muscles of flight undeveloped. In other individuals the muscles of flight and their chitinous supports developed to an intermediate stage, thereby producing the intermediate forms already described. A small proportion only (33.9 per cent.) of the weevils of this species bred in captivity developed perfect muscles of flight. The significance of this remarkable individual variation within the species will be discussed fully after the breeding experiments have been described.

ON THE DEGENERATION OF THE NORMAL MUSCLES OF FLIGHT IN
WEEVILS OF *S. hispidula* AFTER HIBERNATION.

Allusion has already been made to the fact that, when weevils of *S. hispidula* were dissected in the spring, the muscles of flight were found in a much reduced condition, even when the structure of the metathorax was characteristic of a weevil capable of flight. Histologically, however, such muscles differed from the abnormal muscles already described, and it seemed probable that one was here concerned with a degeneration of normal muscle. Confirmation of this view was afforded by finding the muscles of flight in exactly the same reduced condition in individuals

dissected in spring which had been seen to fly the previous autumn. Observations have now been made during 2 years on the condition of flight muscles in weevils of different ages, only those specimens being included in this study which had been observed to fly, or in which the metathorax was found on dissection to be of normal structure. Altogether forty-eight such specimens have been examined from various localities, thirty from North America and eighteen from England. The results showed that while from August to October the muscles were in a functional condition, during the winter (with rare exceptions) they became degenerate, and in the spring were in an entirely atrophied condition. The majority of the insects studied were collected in the autumn, and kept in the insectary throughout the winter, but fresh individuals collected in the spring, in various localities in England, had the fibrous muscles in the same degenerate condition. Great variation was found in regard to the degree of degeneration of the flight musculature in different individuals, even though collected in the same locality and dissected at the same time; and in one specimen collected at Harpenden in October 1927 the muscles were found in a normal condition the following July. Various stages in the regression of the fibrous muscles were observed in the different insects examined, and frequently different fibres in the same insect, or even a single fibre throughout its length, showed different degrees of degeneration. From the data thus obtained the process of degeneration would appear to take place as follows: the fibrils first diminish in thickness and in consequence long spaces occur between them. Instead of being evenly parallel to each other they become undulating and irregular in position. In the same muscle some of the fibrils may be distinct in outline and cross-striated, but in the more degenerate fibrils the edges are blurred and cross-striation has disappeared. Many of the nuclei have become irregular in shape, and where degeneration of the fibrils is advanced the nuclei are no longer arranged in orderly rows. In the next stage the fibrils have completely disappeared, forming an amorphous matrix in which the nuclei are embedded, and the muscle bundles have greatly diminished in size. The nuclei, being no longer subjected to the lateral pressure of the fibrils, have become round or oval in shape and are frequently indented in outline and irregularly placed. Later, there appears to be a sort of pulling together of the disorganised fibre, due probably to the reorganisation of the tracheoles, which have previously been spread out to supply the much greater breadth of the functional muscle. The result is that the fibres become still further diminished in volume (those of the median metathoracic muscle may be reduced to about a quarter of their former

width), and the nuclei in such fibres are of an elongated oval form. At this stage the histology of the muscle shows a superficial resemblance to abnormal (undeveloped) muscle, as sometimes the nuclei may be placed end to end so as to form short chains. This, I consider, is due to the shrinkage of the muscle bundle crowding the nuclei together and not to proliferation of the old muscle nuclei. The nuclei in the degenerated muscle of most of the insects dissected are principally of elongated oval shape (see Plate XXXVI, fig. 6). They are of variable length and are frequently truncated at the end. They can easily be distinguished from the nuclei occurring at the base of the tracheoles as the latter nuclei are shorter, frequently triangular in shape, and stain much more deeply than the muscle nuclei. When such degenerate fibres are examined fresh in physiological salt solution or in dilute glycerine, they appear at first sight to consist of little else but tracheal tubes, so abundant are the tracheae and tracheoles which originally supplied the normal muscle and are now compressed within a much smaller space. If such preparations are stained with methylene blue the nuclei of the muscle become visible but no trace of fibrils can be seen. In fixed preparations, either whole mounts or sections, such degenerate muscle always shows a fine longitudinal fibrillation, just as in the abnormal muscle already described, and this appearance I attribute to the numerous parallel-running tracheoles. The membrane which surrounds each fibre persists so that the fibres retain their individuality. Examined with low magnification the muscle fibres in their final stage of degeneration appear as narrow semi-transparent bands of a glossy whitish appearance. The metathorax of such insects is usually full of fat body, which forms, together with the remains of the median metathoracic muscles, a sort of web beneath the metatergum.

The most striking feature of the degeneration of the fibrous muscles of *Sitona* is that only the fibrils disappear, the nuclei persist. Janet (1907 a) shows that in the degeneration of the muscles of flight of the ant queen, both fibrils and nuclei undergo histolysis. He also describes the penetration of the leucocytes within the persistent envelope of the muscle and their evolution there into adipocytes. In *Sitona* this phenomenon has not been observed; leucocytes, though quite frequent around the degenerating muscle fibres, have not been found within the membrane of the fibre.

The small tubular muscles of flight, which in *Sitona* run from the prescutal muscle disc to the base of the wing process of the pleurum, and from the outer side of the pronator muscle disc to the wing base, have been found in perfect condition in weevils in which the fibrous muscles of

flight are degenerate. In the ant queen Janet (1907 b) finds that the muscles of this type (the muscles "de mise en place des ailes") also undergo histolysis subsequent to the nuptial flight, but much more slowly than do the vibratory muscles. While the latter may be completely histolysed within 32 days of the nuptial flight, he found some of the fibres of the tubular muscles still almost intact in a queen 10 months after flying.

ON THE CONDITION OF THE FLIGHT MUSCLES IN OLD WEEVILS
OF *S. LINEATA*.

The following remarks apply only to those weevils in which the muscles of flight and their chitinous supports have attained their full development; it has already been shown that individuals with the flight musculature undeveloped are very rare in this species.

The imagines of *S. lineata* emerge principally during July and August and the muscles of flight normally attain their full development within about 3 weeks of emergence. In the spring, after hibernation, all the specimens so far examined have had the flight muscles in perfect condition, and the weevils fly readily at this time of year. In the middle of June thirty specimens collected in Suffolk were dissected. Of these seventeen were found to have the flight muscles still fully developed, in four the muscles were reduced in volume and in the remainder they were degenerate. As late as the end of July a weevil of this species has been seen to fly. During the summer the weevils normally die off, but in captivity some specimens have lived beyond their normal span. In such old specimens much variation has been found in the condition of the muscles of flight. Some dissected in August and September of the year following their emergence had the muscles of flight quite normal, but in other specimens they were degenerate. One weevil, collected in Suffolk on August 7th, 1925, was found when killed on August 18th, 1927, to have the flight muscles well developed, though more than 2 years old.

It is evident that in *S. lineata* the muscles of flight are functional for a much longer period than is usually the case in *S. hispidula* and this fact has an interesting corollary with the life histories of the two species (see p. 736). The weevils of *S. hispidula* establish themselves in fields of clover in the autumn and have no further need to migrate, but the weevils of *S. lineata* migrate in spring to crops of peas and beans, and the power of flight is doubtless a great advantage to them during this migration. There is in both species a marked individual variation in regard to the length of time for which the muscles of flight remain functional, and it is probable that with *S. hispidula* natural selection will not operate to

preserve the capacity of flying after hibernation to the same extent as with *S. lineata*.

In case it may be suggested that, in *Sitona*, conditions of captivity may induce a degeneration of the flight muscles, it is of interest to note that not only (as in the case of *S. hispidula*) have specimens with degenerate flight muscles been taken in the field in spring, but conversely with *S. lineata* specimens which had been kept in tumblers since their emergence in the autumn were found when dissected in the following May to have the wing muscles of normal size, and one liberated out of doors was seen to fly away. The question of the supposed effects of disuse on the muscles of arthropods is fully discussed by Poisson (1924, pp. 239-41). He recounts his observations regarding the muscles of hibernated flies and also of macropterous *Gerris*, the wings of which he had removed to ensure the immobility of the vibratory muscles during hibernation. He concludes that the muscle fibre of an arthropod, which has evolved to transverse striation, retains its principal characteristics of structure, even if it remains non-functional for a longer or shorter period, unless it becomes the seat of pathological processes determining its degeneration. Janet (1907 *a*), moreover, considers that, in the histolysis of the vibratory muscles of the ant queen, the bionecrosis sets in too rapidly after the nuptial flight for it to be a question of actual ontogenetic non-functioning. His conclusion that "Il s'agit ici, en réalité, d'un stade ontogénique héréditairement acquis par suite de la persistance phylogénique de non fonctionnement" would seem, however, to relegate the problem into the obscurity of Lamarckism.

BREEDING EXPERIMENTS WITH *S. HISPIDULA* IN RELATION TO THE NORMAL AND THE FLIGHTLESS FORM.

It is obvious that serious difficulties beset any attempt to discover whether the abnormal development of the wing muscles and their chitinous supports is a genetic character or one dependent on environmental conditions for its manifestation. The nature of the metatergum and the wing muscles cannot be ascertained till the weevil has been killed, nor can failure to fly be taken as a criterion of abnormal development of these muscles and their apodemes, since, as already described, the muscles of flight of the normal insects undergo degeneration in spring, and such weevils become as incapable of flight as those individuals in which the muscles have never attained full development. Moreover, both forms of the species may occur in the same locality, and though there is a tendency for northern insects to be flightless and for those from the south

of England to have fully developed wing muscles, one cannot be certain that collections made in any one locality will contain only the one type of insect. Therefore, if one were to assume that the condition of development of the wing muscles were a genetic character, the difficulty of obtaining material genetically pure with which to start breeding experiments would be practically insuperable. In the investigations made, no attempt has been made to unravel a possible genetic relationship between the two forms of macropterous insects, and the following notes relate to general observations on the breeding of normal and abnormal insects, especially in regard to their seasonal appearance.

Reference has already been made to the breeding experiments conducted to determine the genetical relationship of the macropterous to the brachypterous form of *S. hispidula*. Many of the macropterous insects bred in the course of these experiments were found to be of the flightless variety, with the prephragma and postphragma quite undeveloped and the muscles of the abnormal type. Moreover, in breeding together certain of these macropterous insects (obtained from a mating between a heterozygous brachypterous male and a macropterous female), all the offspring examined (some fourteen specimens) were of the flightless form. The following year an attempt was made to breed from some of these insects in the hopes of obtaining fuller data in regard to the condition of the flight muscles of the progeny, but the males used proved to be sexually immature and fertile eggs were not obtained in time. One of these females, however, yielded fertile eggs when mated to a male which had been collected at Harpenden, and particulars of this mating are recorded under Experiment A.

The other breeding experiments consisted in rearing the eggs of females collected in the field. With such material it is obvious that the matings cannot be controlled, the females being already fertilised at the time of collection. However, it was thought that some useful information could be obtained in regard to the heritability of the flightless condition by examining the metatergum of all the parent specimens available, and comparing the proportions of normal and flightless forms in this and in the succeeding generation.

The results of these experiments are given in Table I, which has been arranged so as to show the proportion of normal, intermediate and flightless forms emerging during fortnightly periods from August to October. Only those specimens are listed in this table which died, or were killed, not less than 3 weeks after emergence from the pupal condition. With *S. lineata* it had been found that a period of about 10 days was necessary

to allow for the normal development of the muscles of flight after emergence, and that in weevils killed earlier than this, the muscles and their apodemes were not fully formed. The great majority of the bred insects were examined when 2 to 3 months old, only nine when about 1 month old and some twenty specimens when over 3 months. The different breeding experiments are indicated by letters, and particulars of the parents used for each will now be given. The investigations were directed principally to the condition of the metatergum. The term *undeveloped* signifies that the metatergum is typical of the flightless form as characterised by the suppression of the pre- and postphragma; *fully developed*, that all the apodemes and muscle discs characteristic of the form with perfect wing muscles are present in a fully developed condition; *intermediate*, that the apodemes and muscle discs show only partial development.

Table I.

Condition of development of the flight muscles and their apodemes in weevils of S. hispidula emerged during fortnightly periods from August 10th to October 19th; weevils from A, B and C bred 1928, those from D and E in 1929.

Exp.	Aug. 10th-24th			Aug. 24th-Sept. 7th			Sept. 7th-21st			Sept. 21st-Oct. 5th			Oct. 5th-19th			Percentage undeveloped
	U.	I.	F.	U.	I.	F.	U.	I.	F.	U.	I.	F.	U.	I.	F.	
A	7	.	.	1	.	.	3	.	.	100
B	.	.	.	1	.	1	6	.	1	.	.	.	1	.	.	80
C	.	.	.	1	.	.	1	.	.	1	100
D	5	4	11	9	1	3	8	.	.	4	57.7
E	1	.	.	8	2	1	17	1	2	10	1	83.7
Total	6	4	11	19	3	5	39	1	3	16	1	.	4	.	.	75

U. = undeveloped; I. = intermediate; F. = fully developed. Further explanations in text.

Exp. A. Parents: Female, undeveloped, bred in captivity on August 2nd, 1927 (in a case which was stocked with eggs of two pairs of bred macropterous weevils, all four undeveloped). Male collected at Harpenden on March 3rd, 1928, fully developed. Eggs were laid between April 28th and June 30th, 1928.

Exp. B. Eighteen macropterous weevils were collected at Harpenden on March 3rd, 1928. Of these ten females were removed to a separate jar on March 25th and the eggs they laid between then and April 14th were used for this experiment. Of the ten females, seven were fully developed, two intermediate and one was lost before examination. Of the remaining eight specimens collected, one male and one female were intermediate and the remaining six males were fully developed. Thus of the total of seventeen collected, 76.47 per cent. were fully developed.

Exp. C. Nine macropterous weevils were collected at Harpenden on October 13th, 1927. Three females were separated from the rest, and the eggs laid by them from April 1st to May 4th were used for this experiment. A large flower-pot (as described in a previous paper (1928)) was used in place of boxes for rearing the larvae in this experiment. The three females were fully developed; of the remaining specimens two males and three females were all fully developed and one male was undeveloped, so that a total of 88.8 per cent. were fully developed.

Exp. D. Parents from Strathroy, Ontario, collected August 15th, 1928. All the weevils examined collected from Strathroy on this date were macropterous. Many were dissected and nearly all were found to be of the form with fully developed muscles of flight. Data were kept regarding the structure of the wing muscles or metatergum in some seventy specimens and 84.28 per cent. were fully developed. Forty weevils were selected at random for observations on oviposition, and it was not until the following spring that it was decided to rear their eggs in order to see if a high percentage of fully developed forms resulted. By this time, a number had died and been discarded without investigation of the metatergum, but twenty-five were examined, of which twenty were fully developed, one intermediate and four undeveloped. The eggs were laid between November 2nd and May 23rd, 1929.

Exp. E. Parents also the Canadian weevils, but the eggs were laid from April 12th to May 23rd, 1929.

DISCUSSION OF RESULTS OF BREEDING EXPERIMENTS.

In considering the results of these experiments one fact stands out prominently, namely, that the percentage of flightless forms amongst the offspring is greatly in excess of that occurring amongst the parents. The proportion of normal to flightless forms may even be reversed in the two generations. Thus in *Exp. E*, in which eggs were reared from Canadian weevils collected at Strathroy, 84.2 per cent. of the weevils examined from this locality were found to be of the type with fully developed muscles of flight, but of the bred weevils only 6.9 per cent. were of this type, 9.3 per cent. were intermediate, while 83.8 per cent. had the wing muscles undeveloped and the prephragma and postphragma unformed. The other experiments show a similar marked increase in the proportion of flightless forms amongst the bred weevils.

A second fact deserving of consideration relates to the time of appearance of the normal insects. It will be seen from Table I that the highest proportion of normal forms occurs amongst the weevils which

emerge in the early season, and that those which emerge towards the end of the autumn are all flightless. Exps. D and E are particularly interesting in this respect, for the eggs for both were obtained from the same batch of Canadian weevils, but the eggs used for "stocking" box E were all laid during April and May, while in D many of the eggs had been laid earlier in the season and the imagines in this box commenced to emerge about a fortnight earlier. In D, 57.7 per cent. of the weevils reared were of the "undeveloped" form, while in E, the proportion of "undeveloped" insects obtained was as high as 83.7 per cent. In both these experiments a number of intermediate forms were reared, so that in D the percentage of normal forms was as high as 31.1 and in E only 6.9.

In this species it is usual for each female to continue to lay eggs for several months. Oviposition in Britain usually commences in autumn, but many more eggs are laid in spring and in early summer. In these experiments the eggs used were laid during periods extending over several weeks. One cannot therefore say whether the late-emerging specimens are those produced from the later-laid eggs or whether such individuals have merely developed more slowly, owing perhaps to the larvae finding themselves in a less favourable position for food. In a large box containing growing clover, the feeding conditions cannot be controlled, and some larvae may chance to wander into an area where the clover roots are less abundant. It is known that a restricted food supply can cause a retardation of development, and it is probable that the individuals which emerge very much later than the majority owe their late appearance to this cause. The connection between lateness of emergence and abnormal condition of the muscles of flight is also shown by the following observations on *S. lineata*, a species which with very rare exceptions is an excellent flier. In rearing this species in the insectary in 1928 the majority of the weevils emerged from the end of August to the middle of September, and all such individuals examined (at a suitable time after emergence) had the wing muscles and their chitinous supports fully developed. A few belated individuals made their appearance in October and November. In one of these, killed 45 days after emergence, the wing muscles were found to be very poorly developed and the prephragma and postphragma were still unformed: while, in another, killed the following March, these phragmata were also absent and the muscles of flight were narrow, and when sectioned were seen to be histologically at an early stage of development. In a field in the neighbourhood of St Andrews some specimens of *S. lineata* were collected in October, 1931, following a wet and cold summer. These specimens appeared to be

newly emerged and, in one dissected at that time, the metatergum was still partially transparent. Eight more were dissected six weeks later, and in four of them the flight muscles and phragmata were quite undeveloped, which shows that, under certain conditions, flightless individuals of *S. lineata* do occur in the field. These were apparently late-emerged specimens whose development may have been affected by the inclemency of the season, and even amongst a few specimens of this species bred in the insectary as early as the beginning of September of the same year several flightless forms were obtained.

In a previous paper (1928) the suggestion was put forward that the flightless condition of the macropterous weevils of *S. hispidula* might be hereditary, since almost all the macropterous specimens examined in the course of breeding experiments (conducted to determine the genetical relationship of the macropterous to the brachypterous form) were of the flightless variety. If this supposition were correct it would be reasonable to expect that, in breeding from a mixed population of normal and flightless individuals, the proportions of the two types would be similar in the two generations, but the recent experiments show that this is not the case, the percentage of flightless forms being very much higher in the insects bred in captivity than amongst the "wild" parents¹. In face of this evidence it seems more probable that the cause of the flightless condition is to be sought in unfavourable environmental conditions. It has already been shown that the flightless individuals are merely those in which the normal post-metamorphic development of the muscles of flight and their chitinous supports has been arrested. The cause or causes of such arrested development are likely to remain obscure until more is known of the conditions necessary for growth and differentiation. Frew's interesting work (1928) on the *in vitro* cultivation of the tissues of the blow-fly, indicates that growth and differentiation of the imaginal discs depends directly or indirectly on the concentration of some substance or substances in the body fluid. When larval body fluid was used as a culture medium no evagination of the imaginal discs was obtained, but when pupal body fluid was substituted the discs of the legs evaginated in the culture and grew into definite segmented limbs. It is possible that the arrested development of the wing muscles and their chitinous supports in

¹ In case it should be suggested that the normal development of the muscles of flight was hindered in the captive insects owing to the latter being confined in tumblers since their emergence from the pupal condition, the reader is referred to the remarks on p. 761 regarding the normal development of the flight muscles in weevils of *S. lineata* kept under similar conditions.

the macropterous weevils of *S. hispidula* may be due to a deficiency of some growth-promoting substance in the body fluid of the weevil, either through an insufficiency of food in the larval state or owing to the effect on the metabolism of unfavourable conditions of temperature or humidity. The specimens emerging late in the season are more likely to have experienced conditions unfavourable to the species, and this would explain the prevalence of flightless forms in the weevils appearing at the end of September and in October. Environmental factors may also be responsible for the occurrence of normal and flightless forms emerging at the same time and in the same breeding box, since, as already explained, it is impossible to rear these root-feeding larvae under identical conditions and a box of growing clover provides a varied environment. It is probable that breeding the insects in captivity may tend to reproduce the conditions which cause the arrest of development of the flight muscles. Thus, the fact that the breeding cages had to be covered with muslin and kept under the wooden roof of the insectary, would prevent the normal amount of sunlight penetrating to the soil in which the larvae were developing, and this may retard the growth of the larvae, for it has been observed that the duration of the larval period is usually longer in captivity than in the field. That such artificial conditions cannot be the sole cause of the production of flightless forms is evident from the occurrence of such flightless forms in the field. In this connection it is of great interest to find that, in the north of Scotland, where the summers are colder and the weevils emerge from the pupal condition later in the season, a very much higher proportion of flightless to normal forms has been taken than in the south. Thus out of ninety-seven macropterous specimens collected so far in England and Wales eighty-five have been normal, ten intermediate and only two abnormal, but in the Scottish Highlands out of twenty-three specimens examined only two have been normal, three intermediate, and the remaining eighteen were all abnormal.

To sum up, the evidence obtained from the results of the breeding experiments and from field observations suggests that each macropterous species which may be capable of flight requires, for the full development of the muscles of flight and their chitinous supports, certain optimum conditions of environment during ontogenetic development. The range of environmental variation within which such development can take place will vary for each species, and for *S. lineata* it will be greater than for *S. hispidula*.

SUMMARY.

In macropterous weevils of *S. hispidula* F. marked variation occurs in regard to the development of the muscles of flight, some insects being able to fly while others are permanently flightless. Both forms are widely distributed and may occur in the same locality.

In the flightless insects the histology of the fibrous muscles is entirely different from that of the normal muscle; the discs of the fibrous muscles are reduced in size and the phragmata of the metatergum are suppressed. Forms intermediate between the two extremes occur.

A study has been made of the post-metamorphic development of the flight muscles in *S. lineata*, a species which is an excellent flier, and it has been found that in the newly emerged weevils the condition of the flight muscles and their chitinous supports closely resembles that of the abnormal form of *S. hispidula*. In *S. lineata*, however, the normal condition is usually attained a short time after emergence.

The flightless macropterous weevils of *S. hispidula* may therefore be regarded as individuals in which the normal post-metamorphic development of the flight muscles and their chitinous supports has been arrested.

In the normal form of *S. hispidula* it has been found that the muscles of flight usually degenerate during the winter, but in *S. lineata* they are functional for a much longer period and weevils of this species fly readily in spring.

Evidence obtained by breeding *S. hispidula* suggests that unfavourable environmental conditions during ontogenetic development may be responsible for the production of the flightless weevils.

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EXPLANATION OF PLATES XXXV AND XXXVI.

PLATE XXXV.

Parts of metatergum of *S. hispidula* F.

- Fig. 1. Prescutum and prephragma of macropterous weevil with fully developed muscles of flight. $\times 90$.
 Fig. 2. Prescutum and rudimentary prephragma of macropterous weevil with abnormal muscles of flight. $\times 90$.
 Fig. 3. Postnotum and postphragma of macropterous weevil with fully developed muscles of flight. $\times 65$. (From same individual as fig. 1.)
 Fig. 4. Postnotum and rudimentary postphragma of macropterous weevil with abnormal muscles of flight. $\times 65$. (From same individual as fig. 2.)

In the above figures the degree of thickness of the chitin is shown by the depth of shading.

PLATE XXXVI.

- Fig. 1. Longitudinal section of three-quarters of the length of an entire bundle of a normally developed indirect flight muscle of *S. hispidula*, from a weevil which emerged from the pupal condition about August 18th, 1929, and was killed on November 7th, 1929. It had been bred from Canadian parents. Fixed formol, stained haemalum and eosin. Section 5μ thick. (Owing to the low magnification, and the extreme fineness of the cross-striation, the latter has been omitted.) $\times 200$.
 Fig. 2. Longitudinal section of three-quarters of the length of an entire bundle of an indirect flight muscle of the abnormal type from a weevil of *S. hispidula* which emerged from the pupal condition about September 25th, 1928, and was killed on May 11th, 1929. In this weevil the prephragma and postphragma were undeveloped. Fixed Bouin, stained iron haematoxylin and eosin. Section 5μ thick. $\times 200$.
 Fig. 3. Longitudinal section of a small portion of a normally developed flight muscle of *S. hispidula* from the same preparation from which fig. 1 was taken. (The dark discs of the fibrils do not show in this section but are distinct in another preparation of the flight muscles of this insect, stained with iron haematoxylin.) $\times 1000$.
 Fig. 4. Longitudinal section of an indirect flight muscle of the abnormal type from a weevil of *S. hispidula* which emerged from the pupal condition about September 16th, 1928, and was killed on December 27th, 1928. Fixed Tower No. 3, stained Ehrlich haematoxylin and eosin. Section 6μ thick. The portion figured represents three-quarters of the width of an entire muscle bundle. $\times 1000$.
 Fig. 5. Longitudinal section of the median portion of an entire bundle of an indirect flight muscle from a newly emerged weevil of *S. lineata*, found in breeding case on September 4th, 1928, and killed same day. Fixed Tower No. 3, stained iron haematoxylin. Section 6μ thick. $\times 1000$.
 Fig. 6. Longitudinal section of a degenerate flight muscle of *S. hispidula*, showing on the left a group of tracheoles entering the muscle and three pear-shaped tracheole nuclei. From a weevil collected Strathroy, Ontario, August 1928, seen to fly on September 18th and killed the following January. Fixed Tower No. 3, stained haemalum and eosin. Section 5μ thick. $\times 1000$.
A.A. anterior arm of postnotum; *A.N.P.* anterior notal wing process; *A.P.* anterior phragma or prephragma; *C.R.* transverse chitinous ridge of prescutum; *L.P.* lateral process of prescutum; *M.D.* muscle disc of *musculus relaxator alae*; *N.* nucleus; *P.A.* posterior arm of postnotum; *P.P.* posterior phragma or postphragma; *S.* sarcosomes; *T.* telophragma; *Tr.* tracheae; *Trl.* tracheoles; *X*, line of attachment of tergal membrane connecting with mesonotum; *Z*, line of apparent attachment of first abdominal tergite, the latter being reflexed over the posterior part of postnotum as explained in the text.

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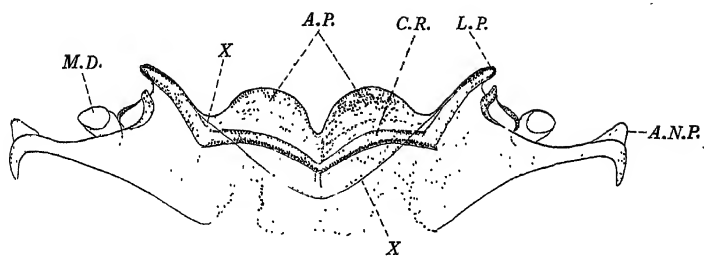


Fig. 1.

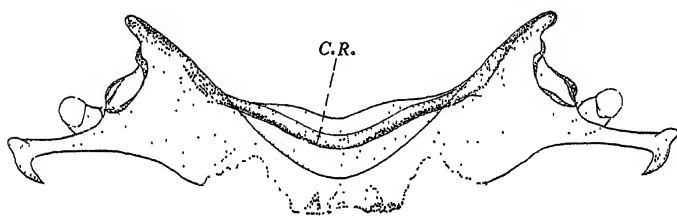


Fig. 2.

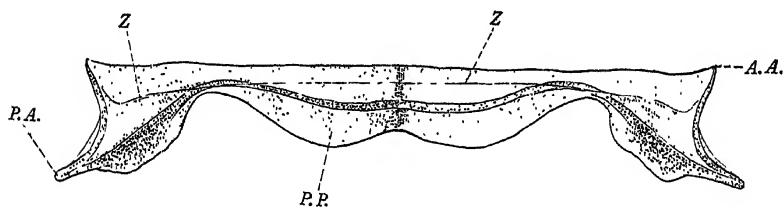


Fig. 3.

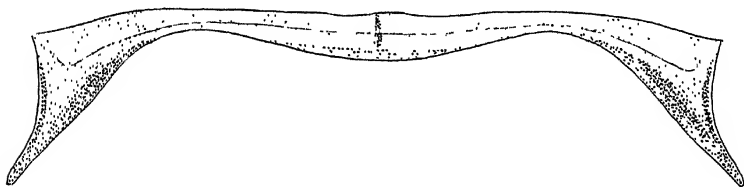


Fig. 4.

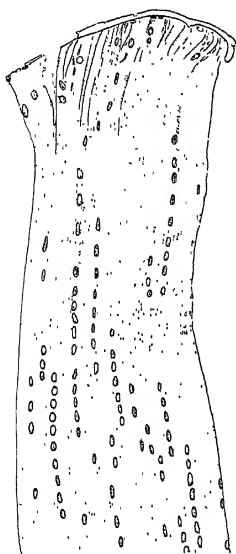


Fig. 1.

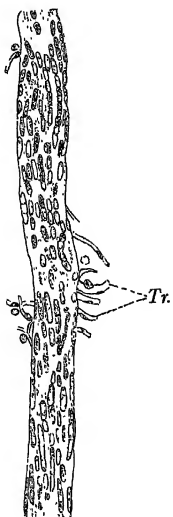


Fig. 2.



Fig. 6.

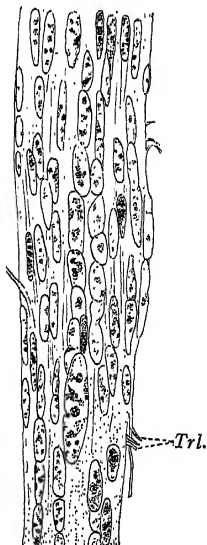


Fig. 5.

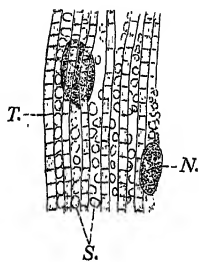


Fig. 3.

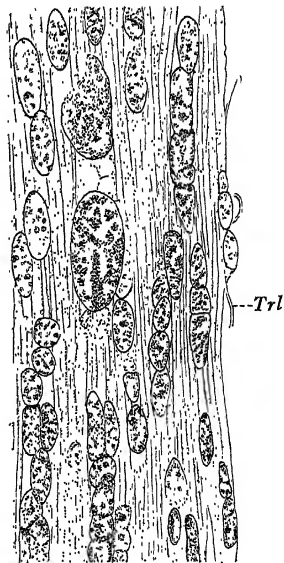


Fig. 4.

THE BREEDING OF *EPHESTIA KÜHNIELLA* ZL. IN LARGE NUMBERS FOR EXPERIMENTAL WORK

By G. H. MANSBRIDGE, M.A.

(Imperial College of Science and Technology, Biological
Field Station, Slough.)

A CONSTANT supply of Stored Products' insects is needed in this station, particularly for experiments on life history and physiology, and very large numbers are used in experiments with fumigants. It is very desirable to have all stages of an insect present at any one time. In the first place an insect was looked for which was important economically and which could be easily bred in large numbers. The Flour Moth, *Ephestia kühniella* Zl., was chosen as being the most suitable insect, but the breeding of it was found to be more difficult than appeared at first sight.

At first an attempt was made to breed this moth under approximately the same conditions as occur in a flour mill. To do this a sack of sweepings from an infested mill was spread with fresh flour about a compartment of one of the insectaries. After a few months great overcrowding occurred, and this was not remedied by the use of trays of food which could be replaced on becoming used up. Numbers of small half-starved larvae swarmed about the room and dwarfed adults appeared. Black larvae, dead from bacterial disease, were present everywhere, and larvae which were apparently healthy turned quite black when brought into a temperature of 27° C. Larvae were also attacked by the hymenopterous parasites, *Nemeritis canescens* and *Microbracon hebetor*, so that they were useless for most experiments. Large numbers of mites (*Cheyletus eruditus*, *Cheyleteomorpha venustissima*, *Typhlodroma* sp. and *Haemogamasus oudeniansi*) were present and they destroyed most of the *Ephestia* eggs.

This method of "mass culture" was of very little use for most experiments, though the moth still bred readily enough in spite of its many enemies. The failure of the method was probably due to the favourable condition presented to the parasites by the great concentration of their host.

As soon as a satisfactory way of breeding *E. kühniella* had been found these "mass culture" stocks were destroyed, being too dangerous as a source of infection to other stocks.

By the co-operation of the members of the Field Station staff a scheme was gradually evolved by which good healthy stocks of *E. kühniella* should be reared. The underlying principle was to have *small isolated cultures in sterilised food and surroundings*. No culture was to be allowed to run for more than one generation.

INITIAL WORK.

The insectaries were fumigated with three heavy doses of HCN, at intervals of about 3 weeks, to get rid of all mites and insects. They were then sprayed with a mixture of formaldehyde and phenol, as a bactericide.

The shelves were covered with very tough brown paper, and any join or hole in this was carefully mended to prevent the passage of mites. The surface of the shelves was now divided into 800 squares by means of strips of lath. Each compartment was 8 in. square and accommodated one tin box, without it touching any of its neighbours.

ISOLATING MEASURES.

A thick skirting of grease-banding was placed round the base of the walls and all round the edges of each shelf thus completely isolating individual shelves. Each tin also had a complete ring of grease-banding round the base. These isolating measures served to prevent the passage, either in or out, of mites and stray insects.

The tins which were to contain the cultures were cylindrical, 8 in. high and 7 in. in diameter. They were made without solder so that they could be sterilised by heat. The lids were without holes and fitted over the edge of the tin, fitting closely enough to keep in large *Ephestia* larvae; small larvae were never noticed to escape from the tins and they would not wander from the flour.

Once these tin cultures had been started, which was done by taking the eggs of carefully selected pairs from the mass culture, the maintenance of them could be reduced to a weekly routine, as follows:

Weekly routine.

1. Sterilising tins for new cultures (in hot oven for 15 min.).
2. Baking flour for new cultures (in slow oven 1-1½ hr.).
3. Collection of pairs in copulation, selecting good pairs by eliminating those which do not break apart within 4 hr.
4. Emptying tins of adults 1 day before pairs are wanted, so as to get young pairs.
5. Placing 6 oz. of baked flour in each tin with a pair of moths.
6. Lettering or numbering of tins and recording the exact date on which the cultures in them were made up.
7. Examination of all the older cultures.
8. Fumigation of the oldest cultures with CS₂ (2 c.c. per tin) and then their destruction.

Cultures which have been made up at the same time are kept together.

Undressed wholemeal wheat flour, coarse ground, is used. This is much cheaper than ordinary wholemeal and *E. kühniella* does very well on it.

The capacity of each tin appears to be about 250 individuals. As a moth frequently produces more progeny than this, crowded conditions sometimes result, this is not a serious drawback as such cultures can usually be used before the larvae are full grown. It might give greater satisfaction, but considerably more work, if, instead of the pair of moths, 250 newly hatched larvae could be used to start each culture, this would also obviate blank cultures, resulting from sterile pairs, not an infrequent occurrence¹.

No particular need for ventilation in the tins has been noticed, though crowded cultures in the warm weather get slightly mouldy by the time the adults are emerging.

In winter when the insectaries are heated, the temperature varies between 15° and 23° C. and the relative humidity is about 60 per cent. From May to September, when there is no artificial heat, the temperature varies considerably. On exceptionally hot days the temperature has reached 28° and 29° C.

Under these conditions *E. kühniella* takes about 12 weeks to complete its life cycle. Accordingly the number of cultures made up each week should be one-twelfth the total number of tins in use. *E. kühniella* fits in well with this routine. Development is fairly regular, the individuals in each tin being all in about the same stage. Any stage can be obtained at very short notice.

Eggs can be procured by placing freshly paired moths in pill-boxes or specimen tubes.

Full-grown larvae are usually found working in the surface layers of the flour, especially at the side of the tin. Quite often large numbers of wandering larvae are found crawling over the inside of the lid.

Younger larvae are more readily found if the eggs are "sown" in one place.

Pupae are found round the side of the tin in the flour and sometimes on the lid.

Newly emerged adults are obtained from tins containing pupae by clearing them of any adults a short time before.

It is a distinct advantage in many experiments to know that the contents of each culture is the progeny of one pair of moths.

¹ See Norris, M. J., *Proc. Zool. Soc.* (in the Press), for an account of sterility in *E. kühniella*.

This method of rearing *E. kühniella* has been in continuous use in this laboratory for more than 2 years. During the last 18 months no mites have been found. At first a few mites were unavoidably introduced on the moths. On the first appearance of mites in a tin a culture was destroyed.

No *Nemeritis* has been seen in the tins. It probably cannot get into a tin with the lid well on.

There was one outbreak of *Microbracon* soon after these cultures were first started. This parasite has a distinct advantage in being able to get in and out of the tins under the lid. By examining all the cultures weekly, any with *Microbracon* were destroyed, and since this has been done there has been no further sign of the parasite in any of the cultures.

The same methods on a smaller scale have been applied to the rearing of other Stored Products' insects in this laboratory. With some of the other insects additional factors such as the need for a high humidity and the occurrence of retarded development in certain individuals also need consideration.

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REVIEWS

Biology in Everyday Life. By JOHN R. BAKER and J. B. S. HALDANE.
G. Allen and Unwin, Ltd., London, 1933. Pp. 123. 3s. 6d. net.

This little book contains a series of wireless talks which have been amended and expanded to make them more suitable for publication. Those by Baker deal with (1) a biologist's view of everyday life, (2) social life in animals, (3) the determination of sex, (4) the quality and quantity of mankind and (5) war, disease and death. In the final chapter Haldane discusses "Biology and statesmanship." Chapter 1, even as an introduction, is rather absurd, but the chapters, fortunately, get progressively better, and contain many ideas and points of view which it is very necessary the general public should appreciate. There are however several statements in Mr Baker's talks for which one might perhaps ask in vain for evidence. A number of references are given as footnotes but these are mostly to particular data and are not suggestive of further reading. For example a reference "See Wheeler" is of as little use to a general reader as is a reference to "O. Salvin. Proceedings of the Zoological Society. 1873" or to "U.S. Dept. Agriculture Bulletin 1078."

Some time ago I was asked by certain educational authorities to write a memorandum on why the general public is not interested in the study of biology. I blamed the way in which biology is usually presented to the general public, but had this book been in print I should have referred to it as the kind of biology which, given a good teacher, would arouse an enthusiastic response in any public audience and lead its members on to further study. In getting such biology as this over to a million listeners broadcasting is doing inestimable service both to science and to social progress. It is very desirable that this little book should have an extensive sale and the great pity is that it was not published at a shilling instead of three shillings and sixpence.

W. B. BRIERLEY.

How Plants get their Names. By L. H. BAILEY. The Macmillan Co.
Pp. 209 with 11 text-figures. 12s. net.

This is a very pleasant book written in a somewhat pedantic style using words such as "estopped" and "obligated." There is a good deal of repetition and some moralising but it is all very nicely done and has caught something of the spaciousness and charm one associates with the mediaeval herbalists. At the same time the book contains a great deal of sound common sense and botanical wisdom, and it is full of quotable passages, e.g. "It is impossible accurately to define what is meant by species. The naturalist gradually acquires the idea and it becomes an unconscious part of his attitude towards living things"; "For horticulturists and botanists alike, the primary problem is not nomenclature but identification... the nomenclature expresses the facts in nature as the particular author interprets them"; "All words are beautiful when properly used and correctly pronounced and relieved of the vulgarisms of slang... accurate clear language is the mark of sensitiveness and intelligence."

The main part of the book concerns the "how" and "why" of plant systematics with interesting and illustrative examples drawn from books and specimens all lying on what must be a perfectly huge table in front of the author. The latter part of the book contains a list of generic names likely to be met with in horticultural literature with indications of accent and vowel quantity and a list of specific names with indication of pronunciation and suggestion of botanical application. The pronunciations are according to American usage, which does not always agree with that of this country, e.g. *Adenanthè-ra* and similarly constructed words, *Apóc-ynum*, *Cygnóc-ladus*, *Heracleù-um*, *Is-atìs*, *Leptóch-ion*, *Lept-ís-yne*, *Leucóc-rinum*, *Ligh-strum*, *Micrós-tylis*,

Resè-da, etc., where a grave accent means a long vowel and an acute accent a short vowel. Minor misprints occur on pp. 38, 64, 70 and 147.

The book will appeal to all plant lovers and form excellent vacation reading for elementary students of botany.

W. B. BRIERLEY.

Traité de Pathologie végétale. By GABRIEL and MADELEINE ARNAUD.
Paris: Paul Lechevalier et Fils, 1932. In 3 volumes (26 × 17 cm.).
Vol. I, pp. 1-993, vol. II, pp. 995-1831, vol. III, atlas of 34 plates in colour. 750 francs.

During the last 50 years numerous treatises have been produced in France dealing with plant diseases, the most notable perhaps being those by Prillieux, Noveu-Lemaire, Marchal and by Delacroix and Maublanc. The largest of these works are the two volumes by the last-named authors, of which the second edition appeared in 1926-7, but even this work appears small beside the volumes now produced by Dr G. Arnaud, joint Director of the Central Phytopathological Station at Versailles and Madeleine Arnaud, his wife. With the exception of Viala's *Les maladies de la vigne* last issued in 1893 the previous French works have been more or less general text-books on plant disease whereas Arnau's volumes are in a different category, being a monographic treatment of the diseases of particular hosts.

The scope and nature of the present work can best be shown by briefly outlining the contents of the three volumes. Following a short preface in vol. I there is a general introductory chapter of 100 pages dealing with the history of plant pathology, the classification of diseases and parasites, the general principles of disease control, the relation of climatic conditions to disease and a bibliography of the principal books on phytopathology. Pages 107-649 are then devoted to a consideration of diseases of the vine and if it had been published as an independent volume this portion alone would rank as a major contribution to phytopathological literature. An historical survey of viticulture in Europe and N. America is followed by sections dealing with the classification and genetics of the vine, the classification of vine parasites and diseases and the control of vine diseases more especially by the use of resistant varieties. The next six sections, comprising 198 pages, deal severally with particular fungal diseases of the vine, each section being arranged in a logical schedule: (a) general considerations, (b) historical account, (c) description of the disease, (d) conditions for the development of the disease, (e) control measures, and (f) mycological and microscopic study. In the next five sections, occupying 76 pages, are considered severally the various fungal diseases of the fruit, leaves, branches, trunk and roots. Seven pages are then devoted to bacterial diseases of the vine and 44 pages to animal parasites and predators. In the final section, comprising 134 pages, are considered the phanerogamic parasites and various diseases of non-parasitic origin. The numerous moieties composing the sections are each followed by bibliographies which are very complete and up to date, in most cases containing references of 1931.

The remainder of vol. I, pages 651-993, is devoted to a consideration of diseases of the apple-tree on lines similar to those described above for the vine and, again, if issued as an independent book this portion alone would rank as a noteworthy contribution.

Vol. II begins with page 995 and the first 150 pages are devoted to diseases of the pear-tree. Then follow shorter chapters dealing respectively with diseases of quince (16 pages), medlar (3 pages), service tree and mountain ash (3 pages), Hawthorn (3 pages), peach and almond (96 pages), apricot (31 pages), plum (85 pages), cherry (33 pages), gooseberry and currant (61 pages), raspberry and blackberry (50 pages), cranberry (7 pages), strawberry (25 pages), olive (44 pages), orange, lemon and citron (57 pages), fig (12 pages), mulberry (53 pages), pomegranate (2 pages), loquat (2 pages), persimmon (2 pages), carob (2 pages), pistachia (2 pages), jujube (1 page), and date-palm (13 pages). Vol. II concludes with a subject-index (52 pages), a table of errata and a table of contents for the three volumes.

Vol. III is an atlas of 34 coloured plates from original paintings by Suzanne Ballings, 13 illustrating diseases of the vine, 5 those of apple, 4 those of pear, 2 each for peach and gooseberry, 1 each for diseases of quince, plum, cherry and apricot, raspberry, olive, fig, mulberry, and a final plate showing fruit rusts on conifers. The plum and some of the vine plates are perhaps a little crude, but in most of the plates the colouring and reproduction are adequate.

The whole work thus runs to some 1900 pages illustrated by 702 text-figures and 34 coloured plates and it is entirely devoted to fruit plants. Although it is essentially a French work on French crops the authors also include in their consideration numerous diseases of these crops only found in other parts of the world.

A few comments may be made on the volumes as a technical work. No reviewer could read these volumes in their entirety and in any case the production is not a book to be read straight through but an encyclopaedia to be constantly referred to. Portions that have been read impress one by the clearness and simplicity of the writing and are characterised by a massive width of learning marshalled in logical sequence and finely tempered by constant reference to factual evidence and personal investigation. The controlling influence of the authors' own researches which illumine the entire treatise is exemplified in the 702 text-figures all of which are from original drawings or photographs. Most of these are excellent, although the rather unusual procedure has been adopted of "touching up" many of the photographs to emphasise salient points or give sharpness to outlines.

Certain minor criticisms of the work may be made. The volumes are far too large and heavy to be bound together in one cover and it would have been more convenient in usage if each volume had possessed its own contents and index instead of these being combined at the end of vol. II. Also it would have been valuable if the literature citations could have been gathered together at the end of the treatise into a single bibliography arranged alphabetically by authors, or if at least an author index had been provided. With the present arrangement there is a considerable waste of time in tracking down particular references. On the other hand a rather surprising feature about these numerous bibliographies is the absence of serious overlapping and duplication, the avoidance of which must have entailed a great amount of labour for the authors. Again, the table of errata refers to many, but by no means all, of the errata in the running text of the volumes, whereas the enormous number of bibliographic references scattered through the book seem to have escaped correction and reek with misprints, noticeable, more especially, in the English and American citations. Few of these bibliographies are free from misprints and some are rather bad, e.g. that on pp. 1656-7 containing 32 citations and 13 misprints or that on pp. 965-6 with 18 citations and 8 misprints.

It is not possible for a reviewer to consider in detail the matter contained in a work built on so epic a scale as this, but it is obvious that every reader with special knowledge must find points on which to differ. I, for example, would like to discuss with the authors their consideration of *Botrytis cinerea*: regarding silver leaf the authors say, "les travaux des auteurs anglais ont détourné l'attention des pathologistes des autres causes, qui au moins au point de vue théorique, ont une plus grande importance"; many American workers will be interested in a consideration of peach yellows and cognate maladies as physiological troubles and so forth almost without end. This kind of thing is, of course, almost inevitable when one or two authors cover a wide field in an intensive manner, but the authors are not afraid of stating their own opinions and bring cogent evidence to support the positions they adopt. Moreover one of the great values which such a personal work as this uniquely affords is that it gives one the opportunity of seeing problems from the different point of view of a worker in another country.

The three present volumes are vols. III, IV and V of the *Encyclopédie Mycologique* published by Lechevalier of Paris and together form volume I of the *Traité de Pathologie végétale*. If it is intended to issue further similar volumes to cover diseases of vegetable, forage and other types of crop, plant pathologists may indeed regard themselves as fortunate. In conclusion, one can only say that the present work is a masterly survey worthy to stand beside the current volumes of Sorauer's *Handbuch*, which it complements in a remarkable way, and that its two distinguished authors have earned the gratitude of all plant pathologists.

W. B. BRIERLEY.

LIST OF MEMBERS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

HONORARY MEMBERS

- APPEL, Geh. Reg.-Rat Prof. Dr OTTO, Biologische Reichsanstalt f. Land- u. Forstwirtschaft, Dahlem, Berlin.
- CHEVALIER, Directeur Aug., Laboratoire d'Agronomie Coloniale, Rue Cuvier 57, Paris (V^e).
- HOPKINS, Dr A. D., Bureau of Entomology, Department of Agriculture, Washington, D.C., U.S.A.
- HOWARD, Dr L. O., late Principal Entomologist, Department of Agriculture, Washington, D.C., U.S.A.
- JONES, Prof. L. R., University of Wisconsin, Madison, U.S.A.
- MARCHAL, Prof. P., Institut National Agronomique, Rue Claude Bernard 16, Paris (V^e).
- NEUMANN, Prof. L. G., École Nationale Vétérinaire, Toulouse, France.
- NILSSON-EHLE, Prof. N. H., University of Lund, Svalöv, Sweden.
- SILVESTRI, Prof. F., R. Scuola Sup. d'Agricoltura di Portici, Naples, Italy.
- VAVILOV, Dr N. I., Director, Institute of Plant Industry, Rue Herzen 44, Leningrad, U.S.S.R.

ORDINARY MEMBERS (*Life Members are marked **)

- 1931 AINSWORTH, G., B.Sc., Ph.C., Experimental and Research Station, Cheshunt, Herts.
- 1920 ALCOCK, Mrs N. L., F.L.S., Royal Botanic Garden, Edinburgh.
- 1928 ALLEN, L. A., M.Sc., Ph.D., The University, Reading, Berks.
- 1908 ASHWORTH, Prof. J. H., D.Sc., F.R.S., Department of Zoology, University of Edinburgh.
- 1932 AUBERTIN, Miss D., M.Sc., British Museum (Natural History), Cromwell Road, London, S.W. 7.
- 1914 BAILEY, M. A., M.C., M.A., Controller of Agricultural Research, Gezira Agricultural Research Service, Shambat, Sudan.
- 1926 BAL, D. V., Agricultural Research Institute, Nagpur, C.P., India.
- 1926 BALFOUR-BROWNE, Prof. F., M.A., Winscombe Court, Winscombe, Somerset.
- 1914 BARKER, Prof. B. T. P., M.A., Director, Research Station, Long Ashton, Bristol.
- 1931 BARNARD, Mrs Dorothea E., c/o B.O.C., No. 102 Bungalow, Khodaung, Upper Burma.
- 1927 BARNES, H. F., B.A., Ph.D., Rothamsted Experimental Station, Harpenden, Herts.
- 1922 BARRATT, Miss K., D.Sc., Principal, Horticultural College, Swanley, Kent.
- 1929 BARRITT, N. W., M.A., Rothamsted Experimental Station, Harpenden, Herts.
- 1931 BARTON-WRIGHT, E., M.Sc., Scottish Society for Research in Plant Breeding, Craigs House, Corstorphine, Edinburgh.

- 1930 BAWDEN, F. C., B.A., Potato Virus Research Institute, School of Agriculture, Cambridge.
- 1923 BAXTER, D. EYRE, Kuala Padah Estate, Bentong, Pahang, Federated Malay States.
- 1932 BEAUMONT, A., M.A., Seale-Hayne Agricultural College, Newton Abbot, Devon.
- 1921 BENNETT, F. T., B.Sc., Ph.D., N.D.A., Agricultural Department, Armstrong College, Newcastle-upon-Tyne.
- 1920 BERRIDGE, Miss E. M., D.Sc., F.L.S., Botany School, Imperial College of Science, London, S.W. 7.
- 1919 BEWLEY, W. F., D.Sc., Director, Experimental and Research Station, Chess-hunt, Herts.
- 1927 BISSETT, N., M.R.C.V.S., University College, Newport Road, Cardiff.
- 1920 BLACKMAN, Prof. F. F., M.A., Sc.D., F.R.S., St John's College, Cambridge.
- 1932 BLACKMAN, G. E., B.A., Jealotts Hill Agricultural Research Station, Warfield Bracknell, Berks.
- 1919 BLACKMAN, Prof. V. H., M.A., Sc.D., F.R.S., Imperial College of Science, London, S.W. 7.
- 1928 BLAKE, R. N. A., M.A., The Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey.
- 1920 BORTHWICK, Prof. A. W., O.B.E., D.Sc., School of Forestry, University of Aberdeen.
- 1919 BRENCHELEY, Miss W. E., D.Sc., F.L.S., F.R.E.S., Rothamsted Experimental Station, Harpenden, Herts.
- 1914 BRIERLEY, Prof. W. B., D.Sc., F.L.S., Dept. of Agricultural Botany, University of Reading, Berks.
- 1914 BROOKS, F. T., M.A., F.R.S., F.L.S., The Botany School, Cambridge.
- 1921 BROOKS, R. ST-JOHN, M.D., M.A., D.P.H., D.T.M. and H., Lister Institute, Chelsea Bridge Road, London, S.W. 1.
- 1933 BROWN, J. M. B., B.Sc., Imperial Forestry Institute, Oxford.
- 1930 BROWN, R., B.Sc., Seale-Hayne Agricultural College, Newton Abbot, Devon.
- 1924 BROWN, Prof. W., M.A., D.Sc., Imperial College of Science, London, S.W. 7.
- 1924 BUCKHURST, A. S., A.R.C.S., D.I.C., Pathological Laboratory, Milton Road, Harpenden, Herts.
- 1920 BUDDIN, W., M.A., Laboratory of Plant Pathology, The University Reading, Berks.
- 1929 BUNTING, R. H., Stored Products Research Laboratory, Slough, Bucks.
- 1928 BURR, S., M.Sc., Department of Agriculture, The University, Leeds.
- 1928 BUSHEY, L. C., F.R.E.S., F.Z.S., Curator of Insects, Zoological Society of London, Regent's Park, London, N.W. 8.
- 1920 BUTLER, E. J., C.I.E., D.Sc., M.B., F.R.S., F.L.S., Director, Imperial Mycological Institute, Kew, Surrey.
- 1930 CALDWELL, J., B.Sc., Ph.D., Rothamsted Experimental Station, Harpenden, Herts.
- 1928 CALLAGHAN, A. R., B.Sc., Department of Agriculture, Sydney, New South Wales, Australia.
- 1932 CAMPBELL, A. H., Ph.D., The University, Bristol.

- 1928 CANN, F. R., D.I.C., Forest Products Research Laboratory, Princes Risborough, Bucks.
Orig. CARPENTER, Rev. G. H., D.Sc., Keeper, Manchester Museum, The University, Manchester.
- 1927 CARROLL, J., M.Sc., D.I.C., A.R.C.S., Albert Agricultural College, Glasnovin, Dublin, Irish Free State.
- 1929 CARTWRIGHT, K. T. ST GEORGE, M.A., Forest Products Research Laboratory, Princes Risborough, Bucks.
- 1914 CAYLEY, Miss D. M., D.Sc., John Innes Horticultural Institute, Merton, Surrey, S.W. 19.
- 1905 CHANDLER, S. E., D.Sc., F.L.S., Imperial Institute, London, S.W. 7.
- 1925 CHEAL, W. F., Gosmoor Lane, Elm, near Wisbech.
- 1926 CHEESMAN, Prof. E. E., B.Sc., A.R.C.S., Imperial College of Tropical Agriculture, Trinidad.
- 1930 CHESTERS, C. G. C., M.Sc., Department of Botany, The University, Edgbaston, Birmingham.
- 1931 CHIPPIINDALE, H. G., M.Sc., University College of Wales, Aberystwyth.
- 1908 CHITTENDEN, F. J., F.L.S., V.M.H., Royal Horticultural Society, Vincent Square, London, S.W. 1.
- 1921 CHRYSTAL, R. N., D.Sc., Imperial Forestry Institute, Oxford.
- 1905 CORNWALLIS*, F. S. W., Linton Park, Maidstone, Kent.
- 1915 COTTON, A. D., F.L.S., Royal Botanic Gardens, Kew, Surrey.
- 1931 CRAIGIE, J. H., A.B., M.Sc., Ph.D., Dominion Rust Research Laboratory, Winnipeg, Manitoba, Canada.
- 1920 CUNLIFFE, N., M.A., D.Sc., School of Rural Economy, Oxford.
- 1929 CUNNINGHAM, H. S., Ph.D., Vegetable Research Station, Riverhead, Long Island, N.Y., U.S.A.
- 1929 CURTIS, Miss K. M., M.A., D.Sc., F.L.S., Cawthron Institute, Nelson, New Zealand.
- 1920 CUTLER, D. WARD, M.A., F.L.S., Rothamsted Experimental Station, Harpenden, Herts.
- 1927 DADE, H. A., A.R.C.S., Mycologist, Research Branch, Department of Agriculture, Aburi, Gold Coast, W. Africa.
- 1915 DAVIDSON, J., D.Sc., F.L.S., F.R.E.S., Waite Agricultural Research Institute, University of Adelaide, Glen Osmond, S. Australia.
- 1927 DAVIES, W. MALDWYN, Ph.D., University College, Memorial Buildings, Bangor, N. Wales.
- 1930 DAWSON, R. B., M.Sc., F.L.S., Director, St Ives Research Station, Bingley, Yorkshire.
- 1923 DIXON*, Miss A., M.Sc., F.R.M.S., Rothamsted Experimental Station, Harpenden, Herts.
- 1930 DONCASTER, J. P., B.A., School of Agriculture, Cambridge.
- 1920 DOWSON, W. J., M.A., D.Sc., Botany School, Cambridge.
- 1920 DRUMMOND, Prof. J. M., M.A., F.L.S., Botany School, The University, Manchester.
- 1923 DU PORTE, ERNEST MELVILLE, M.Sc., Ph.D., F.R.E.S., F.R.M.S., Macdonald College, Montreal, Canada.

- 1925 DURHAM, H. E., Sc.D., M.B., B.S., F.R.C.S., "Dunelm," Hereford.
- 1930 EASTERBY, H. T., Bureau of Sugar Experiment Stations, Brisbane, Queensland, Australia.
- 1928 EASTHAM, Prof. L. E. S., M.A., M.Sc., The University, Sheffield.
- 1927 EDWARDS, E. E., M.Sc., Harper Adams Agricultural College, Newport, Salop.
- 1925 EKINS, Miss E. H., B.Sc., Principal, The College, Studley, Warwickshire.
- 1919 ELLIOTT, Mrs J. S. BAYLISS, D.Sc., Botany School, The University, Birmingham.
- 1922 ESDAILE, Miss P. C., D.Sc., F.Z.S., King's College of Household and Social Science, Campden Hill Road, London, W. 8.
- 1927 EVERETT, J., B.A., Canterton Cottage, Lyndhurst, Hants.
- 1920 FAHMY, T., Mycological Division, Plant Protection Section, Ministry of Agriculture, Giza, Cairo, Egypt.
- 1920 FENTON, E. WYLLIE, M.A., B.Sc., F.R.E.S., Biological Department, Edinburgh and East of Scotland College of Agriculture, 13, George Square, Edinburgh.
- 1929 FINDLAY, W. P. K., M.Sc., A.R.C.S., D.I.C., Forest Products Research Laboratory, Princes Risborough, Bucks.
- 1919 FISHER, K., The School, Oundle.
- 1923 FISHER, R. C., B.Sc., Ph.D., Forest Products Research Laboratory, Princes Risborough, Bucks.
- 1931 FOISTER, C. E., B.A., Ph.D., Royal Botanic Garden, Edinburgh.
- 1920 FOX-WILSON, G., N.D. Hort., F.L.S., F.R.E.S., R.H.S. Laboratories, Wisley, Ripley, Surrey.
- 1913 FRYER, J. C. F., O.B.E., M.A., F.R.E.S., Pathological Laboratory, Milton Road, Harpenden, Herts.
- 1933 GADD, C. H., D.Sc., Tea Research Institute, Talawakelle, Ceylon.
- 1918 GAHAN, C. J., M.A., D.Sc., F.R.E.S., Natural History Museum, S. Kensington, London, S.W. 7.
- 1930 GALLOWAY, L. D., M.A., Shirley Institute, Didsbury, Manchester.
- 1914 GARDINER, Prof. J. S., M.A., F.R.S., Bredon House, Selwyn Gardens, Cambridge.
- 1927 GIBSON, W. H., O.B.E., D.Sc., F.I.C., F.Inst.P., The Linen Industry Research Association, The Research Institute, Lambeg, Co. Antrim, Northern Ireland.
- 1920 GIMMINGHAM, C. T., O.B.E., F.I.C., F.R.E.S., The Pathological Laboratory, Milton Road, Harpenden, Herts.
- 1920 GLYNNE, Miss M. D., M.Sc., F.L.S., Rothamsted Experimental Station, Harpenden, Herts.
- 1920 GOODEY, T., D.Sc., Institute of Agricultural Parasitology, Winches Farm, Hatfield Road, St Albans.
- 1922 GOODWIN, W., M.Sc., Ph.D., S.E. Agricultural College, Wye, Kent.
- 1908 GOUGH, G. G., B.Sc., 45, Poplar Avenue, Edgbaston, Birmingham.
- 1929 GRAINGER, J., Ph.D., Department of Botany, The University, Leeds.
- 1921 GRAY, Prof. P. H. H., M.A., Macdonald College, Quebec, Canada.
- 1929 GREEN, D. E., M.Sc., R.H.S. Laboratories, Wisley, Ripley, Surrey.
- 1933 GREGOR, J. W., Ph.D., Scottish Plant Breeding Station, Corstorphine, Edinburgh.
- 1933 GRIEVE, B. J., M.Sc., Ph.D., D.I.C., Botany School, University of Melbourne, Victoria, Australia.

- 1921 GRUBB, N. H., M.Sc., East Malling Research Station, East Malling, Kent.
- 1920 GWYNNE-VAUGHAN, Prof. Dame HELEN, D.B.E., D.Sc., LL.D., F.L.S., Birkbeck College, Chancery Lane, London, E.C. 4.
- 1920 HALKET, Miss A. C., B.Sc., Bedford College, Regent's Park, London, N.W.
- 1930 HALL, W. J., D.Sc., A.R.C.S., F.R.E.S., Director, Citrus Experimental Station, P.O. Manga, S. Rhodesia, Africa.
- 1924 HARRIS, R. V., East Malling Research Station, East Malling, Kent.
- 1927 HATTON, R. G., M.A., Director, East Malling Research Station, East Malling, Kent.
- 1928 HERFORD, G. V. B., B.A., Biological Field Station, Slough, Bucks.
- 1933 HEY, G. L., M.A., East Malling Research Station, East Malling, Kent.
- 1920 HILEY, W. E., M.A., F.L.S., Dartington Hall Ltd., Totnes, Devon.
- 1920 HILL, Sir A. W., K.C.M.G., M.A., Sc.D., F.R.S., F.L.S., Director, Royal Botanic Gardens, Kew, Surrey.
- 1920 HISCOX, Miss E. R., B.Sc., National Institute for Research in Dairying, Shinfield, Nr. Reading.
- 1933 HOBBY, B. M., B.A., The Hope Department, University Museum, Oxford.
- 1924 HOCKEY, J. F., B.S.A., Pathologist in Charge, Plant Pathology Laboratory, Kentville, Nova Scotia, Canada.
- 1931 HOGGAN, Miss I. A., B.A., M.Sc., Ph.D., Horticulture Building, University of Wisconsin, Madison, Wisconsin, U.S.A.
- 1920 HOLDEN, Prof. H. S., D.Sc., F.R.S.E., F.L.S., Department of Biology, University Park, Nottingham.
- 1919 HORNE, A. S., D.Sc., F.L.S., F.G.S., Botany School, Imperial College of Science, London, S.W. 7.
- 1920 HORTON, E., B.Sc., F.I.C., 10, Crieff Road, Wandsworth Common, London, S.W. 18.
- 1927 HOWES, F. N., M.Sc., Royal Botanic Gardens, Kew, Surrey.
- 1928 HUGHES, A. W. McKENNY, D.I.C., Natural History Museum, S. Kensington, London, S.W. 7.
- Orig. IMMS, A. D., M.A., D.Sc., F.R.S., F.L.S., F.R.E.S., Zoological Laboratory, The Museums, Cambridge.
- 1918 JACKSON, Miss D. J., F.L.S., F.R.E.S., North Cliff, St Andrews, Fife, Scotland.
- 1927 JACOBS, S. E., Ph.D., Bacteriology Department, Imperial College of Science, London, S.W. 7.
- 1927 JAMES, H. C., D.Sc., c/o Agriculture Department, Scott Laboratories, Nairobi, Kenya, Africa.
- 1928 JARETT, Miss P. H., M.Sc., Laboratory of Plant Pathology, Canberra, Australia.
- 1927 JARY, S. G., B.A., S.E. Agricultural College, Wye, Kent.
- 1931 JONES, A. P., M.Sc., Department of Agriculture, The University, Leeds.
- 1927 JONES, G. H., M.A., Plant Protection Section, Ministry of Agriculture, Cairo, Egypt.
- 1927 JOSEPH, E. G., B.Sc., 23, Clanricarde Gardens, London. W. 2.
- 1919 KANNAN, KUNHY, M.A., F.R.E.S., Assistant Entomologist, Government of Mysore, Bangalore, S. India.
- 1927 KEEBLE, Sir FREDERICK, C.B.E., M.A., Sc.D., F.R.S., Hammels, Boars Hill, Oxford.

- 1920 KIDD, F., M.A., D.Sc., Low Temperature Research Station, Downing Street, Cambridge.
- 1907 KING, H. H., F.L.S., F.R.E.S., Government Entomologist, Wellcome Tropical Research Laboratories, Khartoum, Sudan.
- 1907 KING, Prof. L. A. L., M.A., West of Scotland Agricultural College, 6, Blythwood Square, Glasgow.
- 1926 KINGSTON, H. T., 299, Hertford Road, Waltham Cross, Herts.
- 1924 KNIGHT, R. C., D.Sc., Assistant Director, East Malling Research Station, East Malling, Kent.
- 1933 KRAMER, L. M. J., M.A., Ph.D., Gresham's School, Holt, Norfolk.
- 1921 LACEY, Miss M. S., M.Sc., Botanical Department, Imperial College of Science, London, S.W. 7.
- 1921 LAURIE, Prof. R. D., M.A., F.Z.S., Department of Zoology, University College of North Wales, Aberystwyth.
- 1928 LAWRENCE, E., N.D.A., Department of Agriculture, Port Herald, Nyasaland, British E. Africa.
- 1933 LEACH, R., B.A., A.I.C.T.A., 7, Wellington Square, Cheltenham.
- 1926 LE PELLEY, R. H., Ph.D., Department of Agriculture, Nairobi, Kenya, Africa.
- 1920 LLOYD, LLEWELLYN, D.Sc., Zoological Department, The University, Leeds.
- 1928 LYALL, Miss E. M., B.Sc., Imperial College of Science, London, S.W. 7.
- 1931 MCCLEAN, A. P. D., M.Sc., Natal Herbarium, Berea, Durban, Natal, Union of S. Africa.
- 1914 MCCLELLAN, F. C., C.B.E., M.R.A.C., F.L.S., Director of Agriculture, Zanzibar. Orig. MACDOUGALL*, Prof. R. S., M.A., D.Sc., F.R.S.E., F.R.E.S., 9, Dryden Place, Edinburgh.
- 1929 MACGILL, Miss E. I., M.Sc., Department of Zoology, The University, Manchester.
- 1927 MCLENNAN, Miss ETHEL, D.Sc., Botanical Department, The University, Melbourne, Australia.
- 1925 MACLEOD, D. J., M.A., Officer in Charge, Dominion Plant Pathological Laboratory, Fredericton, New Brunswick.
- 1909 MANGAN, Prof. J., M.A., 3, Ceiriog Close, Penarth, Glamorgan, S. Wales.
- 1920 MANGHAM, Prof. S., M.A., University College, Southampton.
- 1917 MANN, H. H., D.Sc., F.L.S., Woburn Experimental Station, Aspley Guise, Bedfordshire.
- 1914 MARSHALL, Sir GUY A. K., D.Sc., C.M.G., F.R.S., F.Z.S., F.R.E.S., Director, Imperial Institute of Entomology, Natural History Museum, London, S.W. 7.
- 1930 MARTIN, H., M.Sc., A.R.C.S., S.E. Agricultural College, Wye, Kent.
- 1922 MASON, E. W., M.A., M.Sc., Imperial Mycological Institute, Kew, Surrey.
- 1920 MASON, F. A., F.R.M.S., 29, Frankland Terrace, Leopold Street, Leeds.
- 1927 MASSEE, A. M., F.R.E.S., East Malling Research Station, East Malling, Kent.
- 1920 MATTICK, A. T. R., B.Sc., Ph.D., National Institute for Research in Dairying, Shinfield, Nr. Reading.
- 1933 MEIKLEJOHN, Miss J., B.Sc., Ph.D., Rothamsted Experimental Station, Harpenden, Herts.
- 1932 METCALFE, Miss M. E., B.Sc., Ph.D., 20, Llantwit Road, Treforest, Glamorganshire.

- 1923 MILES, H. W., M.Sc., Ph.D., Department of Agricultural Entomology, Victoria University, Manchester.
- 1929 MILLAR, A., B.Sc., Seed Testing and Plant Registration Station, East Craigs, Corstorphine, Edinburgh.
- 1921 MILLARD, W. A., D.Sc., Department of Agriculture, The University, Leeds.
- 1932 MISRA, A. B., D.Sc., Ph.D., F.Z.A., F.R.E.S., Department of Zoology, Benares Hindu University, India.
- 1932 MOORE, Miss E. S., B.Sc., Ph.D., Tobacco Research Laboratory, Balfour, C.P., S. Africa.
- 1928 MOORE, W. C., M.A., Plant Pathological Laboratory, Milton Road, Harpenden, Herts.
- 1922 MORLAND, D. M. T., M.A., Rothamsted Experimental Station, Harpenden, Herts.
- 1920 MORRIS, H. M., M.Sc., Agricultural Department, Nicosia, Cyprus.
- 1920 MOSLEY, F. O., F.L.S., Pathology Laboratory, Messrs Lowe and Shawyer, Uxbridge, Middlesex.
- 1925 MUMFORD, E. PHILPOTT, B.Sc., "Old Romney," Beaconsfield.
- 1914 MUNRO, Prof. J. W., M.A., D.Sc., Entomology Department, Imperial College of Science, London, S.W. 7.
- 1919 MURPHY, A. J., 2, Dorset Square, London, N.W. 1.
- 1920 MURPHY, Prof. P. A., D.Sc., B.A., Albert Agricultural College, Glasnevin, Dublin, Irish Free State.
- 1925 MUSKETT, A. E., B.Sc., A.R.C.S., The Queen's University, Belfast, N. Ireland.
- 1928 NEL, R. I., B.Sc., M.Sc., Naga Hoeta Estate, Pematang Siantar, Sumatra (East Coast).
- 1927 NELSON, A., Ph.D., B.Sc., Royal Botanic Garden, Edinburgh.
- 1928 NEWTON, H. C. F., B.Sc., A.R.C.S., D.I.C., Rothamsted Experimental Station, Harpenden, Herts.
- 1930 NORMAN, A. G., D.Sc., F.I.C., Rothamsted Experimental Station, Harpenden, Herts.
- 1920 NOWELL, W., D.I.C., F.L.S., Director, East African Agricultural Research Station, Amani, Tanga, Tanganyika, E. Africa.
- 1928 NUTMAN, J. F., B.Sc., A.R.C.S., East African Agricultural Research Station, Amani, Tanga, Tanganyika, E. Africa.
- 1923 OGILVIE, L., M.A., M.Sc., Research Station, Long Ashton, Bristol.
- 1925 OLDEHAM, J. N., B.Sc., Ph.D., Institute of Agricultural Parasitology, Winches Farm, Hatfield Road, St Albans, Herts.
- 1919 PAINE, Prof. S. G., D.Sc., F.I.C., Imperial College of Science, London, S.W. 7.
- 1930 PARKER, W. H., M.C., M.A., Director, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.
- 1928 PARKIN, E. A., B.Sc., Forest Products Research Laboratory, Princes Risborough, Bucks.
- 1914 PETHERBRIDGE, F. R., M.A., School of Agriculture, Cambridge.
- Orig. PETHYBRIDGE, G. H., M.A., M.R.I.A., Ph.D., B.Sc., Pathological Laboratory, Milton Road, Harpenden, Herts.
- 1928 PICKLES, A., B.Sc., Imperial College of Tropical Agriculture, Trinidad, B.W. Indies.

- 1915 PORTER*, A., D.Sc., Department of Zoology, McGill University, Montreal, Canada.
- 1907 POULTON*, Prof. E. B., M.A., D.Sc., LL.D., F.R.S., Wykeham House, Oxford.
- 1919 PRAIN*, Sir DAVID, Lt.-Col., C.M.G., C.I.E., M.A., M.B., F.R.S., LL.D., F.R.S.E., V.M.H., The Well Farm, Warlingham, Surrey.
- 1923 PRESTON, N. C., B.Sc., Harper Adams Agricultural College, Newport, Salop.
- 1928 RAMSBOTTOM, J., O.B.E., M.A., F.L.S., British Museum (Natural History), Cromwell Road, London, S.W. 7.
- 1931 REICHERT, I., Ph.D., Chief Plant Pathologist, P.O. Box 15, Rehoboth, Palestine.
- 1921 RICHARDS, E. H., B.Sc., F.I.C., Rothamsted Experimental Station, Harpenden, Herts.
- 1928 RICHARDS, O. W., M.A., Department of Entomology, Imperial College of Science, London, S.W. 7.
- 1921 ROACH, W. A., B.Sc., A.R.C.S., D.I.C., A.I.C., East Malling Research Station, East Malling, Kent.
- 1914 ROBERTS, A. W. RYMER, M.A., F.R.E.S., Molteno Institute for Research in Parasitology, Cambridge.
- 1923 ROBINSON, D. H., B.Sc., Harper Adams Agricultural College, Newport, Salop.
- 1918 ROBSON, R., Institute of Agriculture, Chelmsford.
- 1920 ROEBUCK, A., N.D.A., Midland Agricultural College, Sutton Bonnington, Loughborough, Derby.
- 1929 ROGERS, W. S., B.A., East Malling Research Station, East Malling, Kent.
- 1919 RUSSELL, Sir E. JOHN, D.Sc., F.R.S., Director, Rothamsted Experimental Station, Harpenden, Herts.
- 1932 RUSSELL, T. A., B.Sc., A.R.C.S., Department of Agriculture, Paget East, Bermuda.
- 1929 SALAMAN, R. N., M.D., Director, Potato Virus Research Station, School of Agriculture, Cambridge.
- 1914 SALMON, Prof. E. S., F.L.S., S.E. Agricultural College, Wye, Kent.
- 1923 SAMUEL, G., B.Sc., Ph.D., Waite Agricultural Research Institute, University of Adelaide, Glen Osmond, S. Australia.
- 1921 SARGENT, R. H., Technical College, Darlington.
- 1919 SEARLE, G. O., B.Sc., Linen Industry Research Association, The Research Institute, Lambeg, Co. Antrim., N. Ireland.
- 1932 SHARGA, U. S., M.Sc., Ph.D., Agricultural Dept., Etawah, U.P., India.
- 1932 SHEFFIELD, Miss F. M. L., Ph.D., F.L.S., Rothamsted Experimental Station, Harpenden, Herts.
- 1920 SMALL, Prof. J., D.Sc., F.L.S., Queen's University, Belfast, N. Ireland.
- 1928 SMALL, T., M.Sc., A.R.C.S., The States Experimental Farm, Trinity, Jersey.
- 1920 SMITH, E. HOLMES, B.Sc., Botany School, Victoria University, Manchester.
- 1919 SMITH, J. HENDERSON, M.B., Ch.B., B.A., Department of Plant Pathology, Rothamsted Experimental Station, Harpenden, Herts.
- 1920 SMITH, KENNETH M., D.Sc., Ph.D., Potato Virus Research Station, School of Agriculture, Cambridge.
- 1927 SMITH, Prof. N. J. G., M.A., B.Sc., Ph.D., Rhodes University College, Grahams-town (Cape), S. Africa.

- 1913 SOUTH, F. W., M.A., Agricultural Department, Kuala Lumpur, Federated Malay States.
- 1919 SPEYER, E. R., M.A., Experimental and Research Station, Cheshunt, Herts.
- 1920 SPINKS, G. T., M.A., Research Station, Long Ashton, Bristol.
- 1920 STAPLEDON, Prof. R. G., M.A., Director, Imperial Bureau of Plant Genetics, Agricultural Buildings, Alexandra Road, Aberystwyth.
- 1925 STELL, F., Department of Agriculture, Port of Spain, Trinidad, British W. Indies.
- 1921 STENTON, R., F.R.E.S., 22, Milton Road, Harpenden, Herts.
- 1922 STIRRUP, H. H., M.Sc., Midland Agricultural College, Sutton Bonnington, Loughborough, Derby.
- 1919 STONE, H., "Long Reach," Chesterton Fen, Nr. Cambridge.
- 1926 STOREY, H. H., M.A., Ph.D., East African Agricultural Research Station, Amani, Tanga, Tanganyika, E. Africa.
- 1927 STOUGHTON, Prof. R. H., D.Sc., A.R.C.S., The University, Reading, Berks.
- 1933 SUTTON, M. A. F., F.L.S., Sutton and Sons, Ltd. Reading, Berks.
- 1919 TABOR, Prof. R. J., B.Sc., Botany School, Imperial College of Science, London, S.W. 7.
- 1921 TATTERSFIELD, F., D.Sc., F.I.C., Rothamsted Experimental Station, Harpenden, Herts.
- 1914 TAYLOR, H. V., M.B.E., B.Sc., A.R.C.S., Ministry of Agriculture, 10, Whitehall Place, London, S.W. 1.
- 1930 TETLEY, Miss H. U., Low Temperature Research Station, Downing Street, Cambridge.
- 1927 THAYSEN, A. C., Ph.D., Bacteriological Laboratories, Chemical Research Laboratory, Teddington, Middlesex.
- 1931 THOMAS, I., M.Sc., School of Agriculture, Cambridge.
- 1933 THOMSON, J. R., B.Sc., The University, Reading, Berks.
- 1919 THORNTON, H. G., B.A., D.Sc., Rothamsted Experimental Station, Harpenden, Herts.
- 1933 THOROLD, C. A., B.Sc., Department of Agriculture, Nairobi, Kenya.
- 1927 TINCKER, M. A. H., M.A., D.Sc., R.H.S. Laboratories, Wisley, Ripley, Surrey.
- 1930 TOMMERUP, ERIC C., B.Sc., Dunlop Plantations, Ltd., Regent Estate, Batang Malaka, Negri Sembilan, Federated Malay States.
- 1919 TROW, Principal A. H., D.Sc., F.L.S., 9, Clive Crescent, Penarth, Glamorgan.
- 1927 TURNER, W. H., B.Sc., Technical Department, Geo. Monro, Ltd., Waltham Cross, Herts.
- 1913 URICH, F. W., Imperial College of Tropical Agriculture, Trinidad, British West Indies.
- 1915 VAN DER BYL, Prof. P., M.A., D.Sc., F.L.S., University of Stellenbosch, Stellenbosch, Union of S. Africa.
- 1921 VOELCKER, J. A., M.A., B.Sc., Ph.D., F.I.C., Stuart House, 1, Tudor Street, London, E.C. 4.
- 1929 VYVYAN, M. C., M.A., East Malling Research Station, East Malling, Kent.
- 1920 WAKEFIELD, Miss E. M., M.A., F.L.S., Royal Botanic Gardens, Kew, Surrey.
- 1929 WAKELY, C. T. M., B.Sc., Bayer Products, Ltd., 19, St Dunstan's Hill, London, E.C. 3.
- 1932 WALDIE, J. S. L., B.Sc., N.D.A., The University, Reading, Berks.

- 1923 WALKDEN, H., The Raft, Derbyshire Road, Sale, Cheshire.
1931 WALLACE, E. R., B.A., Agricultural Institute, Kirton, Nr. Boston, Lincs.
1928 WALLACE, G. B., B.Sc., Ph.D., Department of Agriculture, Morogoro, Tanganyika Territory, E. Africa.
1919 WALLACE, J. C., Agricultural Institute, Kirton, Nr. Boston, Lincs.
Orig. WARBURTON, C., M.A., Yew Garth, Grantchester, Cambridge.
1919 WARE, W. M., M.Sc., S.E. Agricultural College, Wye, Kent.
1922 WARINGTON, Miss K., M.Sc., Rothamsted Experimental Station, Harpenden, Herts.
1931 WARNER, H., City Health Department, Town Hall, George Street, Sydney, Australia.
1930 WATSON, Mrs M. A., Ph.D., Rothamsted Experimental Station, Harpenden, Herts.
1920 WATT, A. S., Ph.D., B.A., Forestry Department, Cambridge.
1918 WEST, C., D.Sc., A.R.C.S., D.I.C., F.L.S., Low Temperature Research Station, Downing Street, Cambridge.
1923 WESTON, W. A. R. DILLON, M.A., Ph.D., School of Agriculture, Cambridge.
1921 WHITEHEAD, T., D.Sc., A.R.C.S., University College of North Wales, Memorial Buildings, Bangor.
1912 WILLIAMS, C. B., Sc.D., Rothamsted Experimental Station, Harpenden, Herts.
1930 WILLIAMS, P. H., B.Sc., Experimental and Research Station, Cheshunt, Herts.
1919 WILLIS, J. C., M.A., Sc.D., F.R.S., F.L.S., 8, Cavendish Avenue, Cambridge.
1923 WILSON, Miss A. P., A.R.C.S., 116, Fellows Road, London, N.W.
1933 WILSON, A. R., B.Sc., c/o Dr Gregor, North Clermiston House, Cramond Bridge, West Lothian, Scotland.
1914 WILSON, M., D.Sc., A.R.C.S., Royal Botanic Garden, Edinburgh.
1931 WILSON, Prof. S. E., Ph.D., Royal Veterinary College, Camden Town, London, N.W. 1.
1921 WILTSHIRE, S. P., B.A., B.Sc., Assistant Director, Imperial Mycological Institute, Kew, Surrey.
1932 WOOD, J., A.R.C.S., D.I.C., Agricultural Institute, Kirton, Nr. Boston, Lincs.
1926 WOODWARD, R. C., M.A., School of Rural Economy, Parks Road, Oxford.
1914 WORMALD, H., D.Sc., A.R.C.S., East Malling Research Station, East Malling, Kent.
1920 WORTLEY, E. J., F.I.C., M.B.E., F.C.S., Director of Agriculture, St Anns, Port of Spain, Trinidad, British West Indies.
1930 YOUNG, W. H., The Nurseries, Kings Road, Lancing, Sussex.

LAWS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

I. The Association shall be called "The Association of Economic Biologists."

II. The object of the Association shall be to promote the study and advancement of all branches of Biology with especial reference to their applied aspects.

III. The Association shall consist of Ordinary and Honorary Members.

IV. Each candidate for ordinary membership shall be a subject of the British Crown. The nomination form of each candidate for ordinary membership shall bear the signatures of two members and shall be forwarded to the Secretaries. The nomination shall be submitted to the Council and, if approved, the election of the candidate shall be recommended to the Association at the next General Meeting. For the election of any candidate two-thirds of the votes of the members present and voting shall be cast in favour of the candidate.

V. All ordinary members on first election shall pay an entrance fee of half-a-guinea. Ordinary members shall pay an annual subscription of twenty-five shillings, due on January 1st of each year, or may compound for their subscriptions by payment of a sum of twenty-five pounds.

VI. Every member elected to the Association shall receive notice to that effect from the Secretaries and shall continue a member until his written resignation shall be received by the Secretaries, or until his membership be forfeited under the laws. (A member shall be liable for the annual subscription for the year in which his resignation takes effect and, notwithstanding his resignation, shall, if he so desires, receive any subsequent publications of the Association issued during that year.)

VII. Ordinary members shall be entitled to admission to all the meetings of the Association, to vote thereat, to present papers, to take part in discussions, and to receive a copy of the Association's publications. Each member shall be entitled personally to introduce non-members to any General Meeting of the Association. But no member whose subscription is in arrears shall be entitled to vote at a General Meeting or to receive the Association's publications, nor shall any publication be sent to a new member until his entrance fee and subscription shall have been received.

The Council may remove from the roll of the Association any member whose subscription is one year or more in arrears.

VIII. Honorary Members shall be persons, not subjects of the British Crown, who have contributed to an eminent degree to the advancement of the Science of Applied Biology. They shall be recommended by a majority of the whole Council and elected in the same manner as Ordinary Members. The number of Honorary Members shall not exceed twelve and not more than two shall be elected in any one year.

Honorary Members shall each receive a copy of the Association's publications and shall not be liable for the payment of an entrance fee or annual subscription.

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Their privileges shall be the same as those of Ordinary Members except that they shall not be entitled to vote at any election or meeting of the Association.

IX. The business of the Association shall be conducted by a Council consisting of a President, a Treasurer, the Secretaries, of whom there shall be two (one representing the Botanical, the other the Zoological Sections of the Association), the Editors of the Journal, of whom there shall be two, and twelve Ordinary Members. Two members of the Council shall be nominated by the President to act as Vice-Presidents.

X. All properties of the Association, both present and future, shall be deemed to be vested in the Council of the Association for the time being, in conformity with the provisions of the Literary and Scientific Institutions Act, 1854.

XI. The Council shall meet at such times as they may determine; six members shall form a quorum.

XII. The Council shall have the power to fill any vacancies among its number that may occur other than those resulting from the selection for annual retirement from the Council referred to in Law XVII.

XIII. The Council shall have power, at any of their meetings, by two-thirds of the votes of those present and voting, to recommend the removal from the roll of membership of the name of any member for the reason that in their opinion it is contrary to the interests of the Association that he shall remain a member. Such recommendation shall be submitted to the Association at the next General Meeting. ~~For the ejection from the Association of any member two-thirds of the votes of the~~ members present and voting shall be cast in favour of such ejection.

XIV. The Council shall appoint a Publications Committee consisting of the Editors, the Treasurer, two Ordinary Members of the Council, and two Ordinary Members of the Association, who shall be responsible for the publication of the Journal of the Association.

XV. The Council, at a meeting prior to the Annual General Meeting, shall appoint one or more Auditors to audit the Treasurer's accounts.

XVI. The Council shall purchase such books, instruments, specimens, furniture and other necessities as may be required, pass the accounts and authorise their payment, and generally manage the affairs and administer the funds of the Association.

XVII. At a meeting prior to the Annual General Meeting the Council shall nominate a President, a Treasurer, two Secretaries, two Editors, and twelve Councillors, four of whom may not have served during the previous year, for recommendation to the Association for election in the ensuing year. Any member of the Council vacating office shall not be eligible for re-appointment as an ordinary member of Council until after the lapse of twelve months. The list as drawn up by the Council shall be sent to all members resident in Great Britain and Ireland at least four weeks before the date of the Annual General Meeting. It shall be competent for any member, on receipt of the recommendations of the Council, to add the name of a member or members of the Association to the list of candidates for election to the Council; such additional nominations, duly seconded, must be in the hands of one of the Secretaries not less than fourteen days before the Annual General Meeting.

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The Secretaries shall when necessary, and not less than seven days before the Annual General Meeting, issue to every member of the Association resident in Great Britain and Ireland a completed list of the proposed Officers and Council for the year, indicating the names of the proposers and seconders of candidates other than the Council's nominees.

The election of new Officers and Council shall be conducted in the following manner:—At the Annual General Meeting each member present shall receive the list of Officers and Council proposed for the year. If no additional nominations have been received after the Council's recommendations, the list shall be put to the meeting and voted on by a show of hands and the result declared by the Chairman. If additional nominations have been received a ballot shall be taken; each member voting shall hand in person to one of the Secretaries a copy of the list on which has been indicated the names of those candidates whom the member voting desires to serve on the Council. When the ballot has been declared closed the Chairman shall appoint, from among the members present, two persons, not candidates for election, to serve as Scrutineers. In examining the lists handed in the Scrutineers shall set aside and take no account of any ballot paper which supports candidates in excess of the number provided for in Law IX, nor of any ballot paper which indicates the identity of the member voting. The Scrutineers shall report to the Chairman of the meeting the result of their scrutiny, and the Chairman, before the close of the meeting, shall announce the result of the ballot. In the case of an equality of votes for any candidates, the power of selection between them shall rest with the Chairman of the meeting and shall be exercised before announcing the result of the ballot.

XVIII. The Association shall meet at times and places to be decided by the Council.

At all Ordinary General Meetings ten shall form a quorum (see also Law XIX). All meetings shall be announced by circular addressed to each member resident in Great Britain and Ireland. At all Ordinary General Meetings the order of business shall be decided by the Chairman.

An Annual General Meeting shall, unless otherwise decided by the Council, be held on the date of the Ordinary General Meeting falling nearest to the beginning of the year.

At this Annual General Meeting the order of business shall be:

1. The reading of the minutes of the previous meeting.
2. The reading of a report of the Council on the work of the past year.
3. The statement of the Treasurer.
4. The election of Members.
5. The election of Officers and other Members of the Council.
6. Other business.

A Special General Meeting may be called to discuss or take action upon any matter affecting the interests of the Association.

A Special General Meeting shall be called either by the decision of the Council or at the request of at least ten members addressed to the Secretaries.

XIX. No new law shall be passed nor any standing law altered or added to, nor any other change in the constitution of the Association made except by a Special

General Meeting of which for this purpose a fourteen days' notice must be sent to all Members resident in Great Britain and Ireland.

The requisition for such a Special General Meeting duly signed and stating in writing the laws proposed or the alteration desired, must be delivered to one of the Secretaries, who shall within a reasonable period call such a meeting. The proposed new laws or alterations in the laws shall be printed in the circular convening the meeting.

At a Special General Meeting convened for the purpose of altering the constitution or amending the laws, fifteen shall form a quorum and no motion can be passed except by a two-thirds majority of those present and voting.

Note.

The following resolutions have been passed by the Council of the Association:

(1) That manuscripts reporting investigations dealing primarily with proprietary substances of which the composition or nature is not specified in such a way that the investigations can be repeated by other workers be not eligible for publication in the *Annals of Applied Biology*.

(2) That the mention of proprietary substances without specification of nature or composition be permissible in a manuscript provided that the particular substances are well known or standard and are only incidental in the work or contributory or subsidiary to the main theme or are used for purposes of control or comparative experiment.

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